FINAL REPORT

HUMAN CELL LINE ACTIVATION TEST (h-CLAT)

Laboratory Study Number:

18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Study Completion Date:

30 October 2019

Authors:

Rishil J. Kathawala, Ph.D.
Kyrstin Driscoll, B.S.

Sponsor

National Institute of Environmental Health Sciences (NIEHS)
NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
601 Keystone Park Drive, Suite 200
Morrisville, NC 27560

Performing Laboratory:

Institute for In Vitro Sciences, Inc.
30 W. Watkins Mill Road, Suite 100
Gaithersburg, MD 20878

Laboratory Project Number:

10426

TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

The Human Cell Line Activation Test (h-CLAT) of the test substances: ACTICIDE OIT, Vanquish 100, Mergal MITZ, Mergal BIT Technical, KORDEKTM 573F BIOCIDE, KATHON 287T Industrial Microbicide was conducted in compliance with the principles presented in the EPA FIFRA (40 CFR part 160) series on Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test substances or assay controls have not been determined by the testing facility. However, the Sponsor (test substances) and the manufacturers (controls) provided Certificates of Analysis that are included in Appendix C.

The stability of the test substances or assay controls under the storage conditions at the testing facility and under the actual test conditions has not been determined by the testing facility and is not included in the final report.

Analyses to determine the uniformity, concentration, or stability of the test article mixtures, if applicable, were not performed by the testing facility.

Study Director: [redacted] 30 October 2019

Rishil J. Kathawala, Ph.D. Date

QUALITY ASSURANCE STATEMENT

Study Title: The Human Cell Line Activation Test (h-CLAT)

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Study Director: Rishil J. Kathawala, Ph.D.

A random sampling approach was used to select at least one in-process, laboratory phase to inspect for this study. The Quality Assurance Unit inspections specific to this study are listed in the table below. Procedures, documentation, equipment records, etc., were examined in order to assure that the study was performed in accordance with the EPA FIFRA (40 CFR part 160) series on Good Laboratory Practice and to assure that the study was conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected and report dates of QA inspections of this study:

Phase Inspected	Audit Date(s)	Reported to Study Director and Management
Protocol and Initial Paperwork	24 April 2019	24 April 2019
Preliminary Assay- Solvent Selection (18AO64, 19AA12, AA13)	20 May 2019	20 May 2019
Draft Report, Data and Protocol Amendment I	12-13 September 2019 & 16-18 September 2019	18 September 2019
Final Report and Protocol Amendment II & III	24 October 2019 29 October 2019	24 October 2019 29 October 2019

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

[redacted] 30 Oct 2019

Megan Conahan, B.S., RQAP-GLP Date

Quality Assurance

SIGNATURE PAGE

HUMAN CELL LINE ACTIVATION TEST (h-CLAT)

Initiation Date:	23 April 2019
Laboratory Start Date:	20 May 2019
Laboratory Completion Date:	21 August 2019
Completion Date:	30 October 2019
Sponsor:	National Institute of Environmental Health Sciences (NIEHS) NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
Sponsor's Representative:	Judy Strickland, Ph.D., DABT Integrated Laboratory Systems, Inc., Contractor supporting the NICEATM
Testing Facility:	Institute for In Vitro Sciences, Inc. 30 W. Watkins Mill Road, Suite 100 Gaithersburg, MD 20878
Archive Location:	Institute for In Vitro Sciences, Inc. Gaithersburg, MD 20878
Director, Laboratory Services:	Gertrude-Emilia Costin, Ph.D., M.B.A., ATS

Study Director: [redacted] 30 October 2019

Rishil J. Kathawala, Ph.D. Date

TEST SUBSTANCE RECEIPT

IIVS Test Substance Number	Sponsor Designated Synonym^	Trade Name	Lot/Batch Number	Physical Description	Receipt Date	Storage Conditions*
18AO64	OIT	ACTICIDE OIT	MX-183774-2006	clear light yellow non-viscous liquid	18 December 2018	room temperature
19AA05	BBIT	Vanquish 100	Lot# 6445	clear orange semi-viscous liquid	10 January 2019	room temperature
19AA12	CMIT/MIT Mixture	Mergal MITZ	Lot# SLJ0229	clear colorless non-viscous liquid	14 January 2019	room temperature
19AA13	BIT	Mergal BIT Technical	Lot# YL201811073	white powder	14 January 2019	room temperature
19AA98	MIT	KORDEK™ 573F BIOCIDE	Batch# YY00H3A451	clear colorless non-viscous liquid	7 February 2019	room temperature
19AB24	DCOIT	KATHON 287T Industrial Microbicide	YY00H77338	off-white waxy solid	15 February 2019	room temperature

* - Protected from exposure to light

^ - Chemical name for Sponsor designated synonym is as follows:

OIT is also known as 2-n-Octyl-4-isothiazolin-3-one
BBIT is also known as 2-butyl-1,2-benzisothiazolin-3-one; synonym: 1,2-Benzisothiazol-3(2h)-one, 2-butyl
CMIT/MIT is also known as 5-Chloro-2-methyl-4-isothiazolin-3-one/2-Methyl-4-isothiazolin-3-one
BIT is also known as 1,2-Benzisothiazolin-3-one
MIT is also known as 2-Methyl-4-isothiazolin-3-one
DCOIT is also known as 4,5-Dichloro-2-octyl-3(2h)-isothiazolone

INTRODUCTION

The Human Cell Line Activation Test (h-CLAT) was used to assess the skin sensitization potential of the test substance(s) by monitoring the upregulation of cell surface markers, CD54 and CD86, on the surface of human acute monocytic leukemia cells (THP-1). The upregulation of CD54 and CD86 in response to

a skin sensitizer is correlated to dendritic cell activation, which is the third key event of the skin sensitization pathway.

MATERIALS AND METHODS

The assay procedures were performed as outlined in the study protocol (See Appendix A).

DEVIATIONS

A deviation occurred in the definitive trail B6 of the study. Per protocol, following the three rinses of FACS buffer, the cells are to be suspended in 600 microliters of 0.01% (w/v) blocking suspension and incubated at 2-8°C for 15±1 minutes. However, the sample of positive control DNCB stained with anti-FITC isotype antibody was incubated at 2-8°C for 15 minutes and for approximately an additional 40 minutes at room temperature. In addition, the protocol mentions that cells will be suspended in a final addition of 200 microliter of FACS buffer prior to running them on the flow cytometer. Given the limited availability of the sample of positive control DNCB, the cells were resuspended in a final volume of 100 microliters of FACS buffer to have an appropriate density of cells for the flow cytometer reading. This was a deviation from the study protocol, however, given that blocking step majorly involved non-specific binding, this deviation would not be of significant impact.

RESULTS AND DISCUSSION

Solubility Determination

Prior to the preliminary (dose range finding) assay, the test substances were tested in a solubility test to determine an appropriate solvent. The following observations were determined during the solubility test:

The test substance, ACTICIDE OIT, was found to be soluble at 500 mg/mL in DMSO with vortexing for 1 min. The description of the dilution was noted to be a clear colorless non-viscous solution. The test substance dilution was observed to remain in solution in the primary solvent as well as culture medium.

The test substance, Mergal MITZ, was found to be soluble at 500 mg/mL in DMSO with vortexing for 1 min. The description of the dilution was noted to be a clear colorless non-viscous solution The test substance dilution was observed to remain in solution in the primary solvent as well as culture medium.

The test substance, Mergal BIT Technical, was found to be soluble at 500 mg/mL in DMSO with vortexing for 3 min. The description of the dilution was noted to be a clear colorless non-viscous solution The test substance dilution was observed to remain in solution in the primary solvent as well as culture medium.

The test substance, Vanquish 100, was found to be soluble at 250 mg/mL in DMSO with vortexing for 1 min. The description of the dilution was noted to be a clear light yellow non-viscous solution. The test substance dilution was observed to remain in solution in the primary solvent as well as culture medium.

The test substance, KORDEK™ 573F BIOCIDE, was found to be soluble at 250 mg/mL in DMSO with vortexing for 1 min. The description of the dilution was noted to be a clear colorless non-viscous solution. The test substance dilution was observed to remain in solution in the primary solvent as well as culture medium.

The test substance, KATHON 287T Industrial Microbicide, was found to be soluble at 500 mg/mL in DMSO with vortexing for 30 sec. The description of the dilution was noted to be a clear light yellow non-viscous solution. The test substance dilution was observed to remain in solution in the primary solvent as well as culture medium.

The neat test substance, KATHON 287T Industrial Microbicide, was heated in a glass water bath at 53°C on a hot plate (IIVS0967) for ~ 5 minutes immediately prior to addition of the solvent, as per sponsor instructions.

Dose Range Finding Assay

A preliminary (dose range finding assay) was performed to determine the viability of the THP-1 cells after 24 ± 0.5 hour exposure to 8 test substance concentrations. The CV75, which is the concentration leading to 75% cell viability was calculated for each test substance.

Definitive Assays

Based on the results of the dose range finding assay, the doses were chosen for the test substances for the definitive assays. At least two valid definitive trials were performed.

Seven serial doses using a typical dilution factor of 1.2 were prepared such that eight doses were tested in the definitive assay. If there was insufficient cytotoxicity in the dose finding assay (i.e. CV75 > highest prepared dose), the highest soluble concentration of test article, up to a maximum stock concentration of 500 mg/mL in either saline or DMSO was selected.

If the first two independent assays were not concordant, a third assay was performed and the final prediction was based on the mode of the conclusions from the three individual runs (i.e. 2 out of 3).

The positive control, 2,4-Dinitrochlorobenzene, was tested in the definitive assays only.

Evaluation of Test Results

The relative fluorescence intensity (RFI) was calculated for each test substance and control treated cell population. RFI ≥ 200 at any tested concentration for CD54, and/or RFI ≥ 150 at any tested concentration for CD86 was considered a sensitizer by the h-CLAT.

The EC200 and EC150 values, which are the calculated test substance concentrations leading to an RFI of 200 or 150, were calculated for each test substance.

If the RFI of CD86 is equal to or greater than 150 at any tested dose with >50% cell viability in at least

two independent assays and/or if the RFI of CD54 is equal to or greater than 200 at any tested dose with >50% cell viability in at least two independent assays, the prediction will be considered as positive (sensitizer). Otherwise, the prediction will be considered as negative.

Summary

Table 1 presents the results from the Dose Finding Assay.

Table 2 presents the results for the valid definitive trials.

Table 3 presents the results for the positive control (2,4-Dinitrochlorobenzene.)

An assay met acceptance criteria when:

- The cell viability values of the solvent controls were > 90%.
- For the solvent controls, RFI values of both CD86 and CD54 were less than the positive criteria (CD86 RFI < 150 and CD54 RFI < 200).
- For the positive control (DNCB), RFI values of both CD86 and CD54 were predicted to be positive (CD86 RFI ≥ 150 and CD54 RFI ≥ 200), and cell viability was > 50%.
- For the medium and solvent controls, the MFI ratio of both CD86 and CD54 to isotype control was > 105%.
- The cell viability of the test substance-treated cultures was > 50% in at least four doses.

All acceptance criteria for a valid assay were met for the definitive trials presented in this report. The test substances, ACTICIDE OIT, Vanquish 100, Mergal BIT Technical, KORDEK™ 573F BIOCIDE and KATHON 287T Industrial Microbicide, were considered sensitizers according to the h-CLAT. Mergal MITZ was considered a non-sensitizer according to the h-CLAT.

Table 1

Test Substance Results for h-CLAT Dose Range Finding Assay

IIVS Test Substance Number	Sponsor Designated Synonym	CV75 (µg/mL)
18AO64	OIT	8.0
19AA05	BBIT	4.8
19AA12	CMIT/MIT Mixture	31.8
19AA13	BIT	17.8
19AA98	MIT	37.3
19AB24	DCOIT	1.1

Table 2

Test Substance Results for h-CLAT Definitive Assay

IIVS Test Substance Number	Sponsor Designated Synonym	Trade Name	CV75 (µg/mL)	Trial	CD54 EC_{200} (µg/mL)	CD86 EC_{150} (µg/mL)	Sensitization Potential	Overall Sensitization Potential
18AO64	OIT	ACTICIDE OIT	8.0	B1 Assay Date: 11 Jun 2019	<2.7	>9.6[1]	Sensitizer	Sensitizer
				B2 Assay Date: 18 Jun 2019	<2.7	>9.6[1]	Sensitizer	
19AA12	CMIT/MIT Mixture	Mergal MITZ	31.8	B1 Assay Date: 11 Jun 2019	>22.1[1]	>22.1[1]	Non-sensitizer	Non-sensitizer
				B2 Assay Date: 18 Jun 2019	16.91	>18.4[1]	Sensitizer	
				B3 Assay Date: 2 Jul 2019	>18.4[1]	>18.4[1]	Non-sensitizer	
19AA13	BIT	Mergal BIT Technical	17.8	B1 Assay Date: 11 Jun 2019	6.0	>17.8[1]	Sensitizer	Sensitizer
				B2 Assay Date: 18 Jun 2019	<6.0	>17.8[1]	Sensitizer	
19AA05	BBIT	Trade name Vanquish 100	4.8	B3* Assay Date: 2 Jul 2019	<1.6	2.12	Sensitizer	Sensitizer
				B4 Assay Date: 10 Jul 2019	2.13	3.86	Sensitizer	
19AA98	MIT	KORDEK™ 573F BIOCIDE	37.3	B3* Assay Date: 2 Jul 2019	<12	35.87	Sensitizer	Sensitizer
				B4 Assay Date: 10 Jul 2019	16.11	>45[1]	Sensitizer	
19AB24	DCOIT	KATHON 287T Industrial Microbicide	1.1	B3# Assay Date: 10 Jul 2019	0.71	>1.37[1]	Sensitizer	Sensitizer
				B6# Assay Date: 19 Aug 2019	0.84	>1.37[1]	Sensitizer	

*B1 and B2 definitive trials did not meet assay acceptance criteria for positive control and therefore those trials were not considered valid.

[#]B1 trial for 19AB24 did not meet assay acceptance criteria for positive control and therefore those trials were not considered valid. In B2 trial, CD86 and Isotype control antibodies were inadvertently plated in reverse, leading to higher Isotype control values and negative RFI. Therefore, results from this B2 trial were not considered valid. B4 and B5 definitive trials did not meet assay acceptance criteria for positive control and therefore those trials were not considered valid.

[1] - ">" values reflect a negative response (i.e., insufficient induction for a positive response).

Table 3

Positive Control Results for the Definitive Assay

Date	Trial	CD54 RFI	CD86 RFI	Cell Viability (%)	Results
11 Jun 2019	B1	668.50	158.07	78.97	Pass
18 Jun 2019	B2	1442.44	171.78	74.00	Pass
2 Jul 2019	B2/B3	751.74	158.24	83.31	Pass
10 Jul 2019	B3/B4	1050.98	197.64	78.22	Pass
19 Aug 2019	B6	1462.78	166.83	81.26	Pass

APPENDIX A (Protocol, Protocol Attachment 1, Protocol Amendment I, Protocol Amendment II & Protocol Amendment III)

HUMAN CELL LINE ACTIVATION TEST (h-CLAT)

1.0 PURPOSE

The purpose of this study is to identify potential skin sensitizers and non-sensitizers in accordance with the United Nations Globally Harmonized System (UN GHS). The skin sensitization potential of a test article is evaluated by measuring the changes in the expression of cell surface markers CD54 and CD86 associated with the process of dendritic cell activation in the human leukemia cell line, THP-1, following exposure to a test article. The changes of surface marker expression are measured by flow cytometry following cell staining with fluorescently labelled antibodies for CD54 and CD86.

2.0 SPONSOR

2.1 Name: National Institute of Environmental Health Sciences (NIEHS)
NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

2.2 Address: Judy Strickland, Ph.D., DABT
Integrated Laboratory Systems, Inc.,
Contractor supporting the NICEATM

601 Keystone Park Drive, Suite 200
Morrisville, NC 27560
(919) 281-1110 x245
strickl2@niehs.nih.gov

2.3 Representative: Judy Strickland, Ph.D., DABT

3.0 IDENTIFICATION OF TEST ARTICLES AND ASSAY CONTROLS

3.1 Test Article(s): See Protocol Attachment 1

3.2 Assay Controls: Positive: 2,4-dinitrochlorobenzene (DNCB)
(2 mg/mL in DMSO)
Solvent/Vehicle: Saline or Cell Culture Medium for aqueous-soluble or surfactant test articles
Dimethyl Sulfoxide (DMSO) for DNCB and DMSO soluble test articles

3.3 Determination of Strength, Purity, etc.

3.3.1 For GLP studies only, the Institute for In Vitro Sciences, Inc. (IIVS) will attempt to secure documentation of the analytical purity and composition of the test article and the stability and strength of the dosing solutions from the Sponsor. If the Sponsor is unable to provide such information, IIVS will retain documentation

supporting attempts to obtain this information with the study file and an exception will be noted in the Statement of Compliance in the Final Report.

3.3.2 IIVS will be responsible for the documentation of the analytical purity and composition of the controls and solvents used in the assay. This may be accomplished by maintaining a certificate of analysis from the supplier.

3.3.3 The stability of the test article(s) and dosing solutions under the storage conditions at the testing facility and under the actual experimental conditions will not be determined by IIVS.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1	Name:	Institute for In Vitro Sciences, Inc.
4.2	Address:	30 W. Watkins Mill Road, Suite 100 Gaithersburg, MD 20878
4.3	Study Director:	Greg Mun, B.A.

5.0 TEST SCHEDULE

5.1	Proposed Experimental Initiation Date:	29 April 2019
5.2	Proposed Experimental Completion Date:	17 May 2019
5.3	Proposed Report Date:	21 June 2019

6.0 TEST SYSTEM

The h-CLAT is an *in vitro* assay which measures the changes in the expression of cell surface markers CD54 (ICAM-1) and CD86 associated with the process of dendritic cell activation in the human acute monocytic leukemia cell line, THP-1 (American Type Culture Collection, ATCC, Manassas, VA, TIB-202™). Dendritic cell activation is considered one of the key biological events in the adverse outcome pathway for skin sensitization, where CD54 and CD86 are subsequently involved in dendritic cell migration to the lymph nodes and T-cell priming. THP-1 cells, seeded at a density of 2.0×10^6 cells/mL in culture medium in 24-well plate format define the Test System. After treatment of the test or control articles to the Test System, the expression of cell surface markers are measured by flow cytometry following cell staining with fluorescein isothiocyanate (FITC) labelled antibodies. Cytotoxicity measurement, using propidium iodide (PI) staining, is conducted concurrently to assess whether upregulation of surface marker expression occurs at sub-cytotoxic concentrations.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The experimental design of this study consists of a dose range finding assay and at least two definitive assays to determine the changes in the expression of the cell surface markers CD54 and

CD86. The Relative Fluorescence Intensity (RFI) is used as an indicator of CD54 and CD86 expression. RFI is calculated from the Geometric Mean Fluorescence Intensity (MFI) data acquired by flow cytometry software. The flow cytometry data acquisition will be performed using a MACSQuant Analyzer (Miltenyi) with a three laser system capable of both FITC and PI acquisition. The procedures are based on those presented in the OECD Test Guideline 442E and the EURL ECVAM DB-ALM Protocol No. 158.

7.1 Medium and Reagents

7.1.1 Culture Medium (RPMI-1640 with 10% heat inactivated Fetal Bovine Serum and 0.05 mM 2-mercaptoethanol)

7.1.2 Saline (0.9% NaCl)

7.1.3 DMSO, CAS 67-68-5

7.1.4 DNCB, CAS 97-00-7

7.1.5 Calcium and Magnesium Free Dulbecco's Phosphate Buffered Saline (CMF-DPBS)

7.1.6 FACS Buffer (CMF-DPBS with 0.1% (w/v) Bovine Serum Albumin - Fraction V) to be fully dissolved before use

7.1.7 Blocking Suspension (1% w/v globulins- Cohn fraction II, III, Human in FACS Buffer)

7.1.8 FITC Mouse anti-Human CD54, Clone 6.5B5 (DAKO/Agilent)

7.1.9 FITC Mouse anti-Human CD86, Clone FUN-1 (BD Pharmingen)

7.1.10 FITC Mouse IgG1 K Isotype Control (DAKO/Agilent)

7.1.11 PI solution (12.5 µg/mL of propidium iodide in CMF-DPBS)

7.2 Environmental Conditions

Throughout this protocol, ranges for test material and test system exposure or incubation conditions (e.g., temperature, humidity, CO_2) are presented. These ranges describe the equipment performance specifications under static conditions (i.e., in the absence of frequent opening of equipment doors, accessing chambers, changing loads, etc.), as presented in the relevant equipment SOPs.

7.3 Maintenance of THP-1 Cell Line

Cryopreserved THP-1 cells, tested for and cleared of mycoplasma contamination, will be stored in liquid nitrogen. The stock ampule(s) will be thawed and slowly diluted in

approximately 9 mL of culture medium kept at 2-8°C. To wash the cells of cryopreservative, the cells will be collected by centrifugation (200-300g, in a centrifuge set for 5 minutes and 4°C). The rinse will be repeated with the same volume of medium and centrifuge settings. After the second rinse, the cells will be resuspended in an appropriate volume of culture medium warmed to approximately 37°C for the culture vessel used (typically either T25 or T75 flasks without a growth surface). The cells will be maintained at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air (standard culture conditions) with at least one agitation per each day. Cells will typically be refed every 2-3 days with culture medium warmed to approximately 37°C until the cells are confluent enough to be passaged or transferred to a larger culture vessel.

Cells will be routinely passaged every 2 to 3 days and seeded at a density of 0.1×10^6 to 0.2×10^6 cells/mL. The cells will routinely be maintained at densities ranging from 0.1 to 0.8×10^6 cells/mL. The cell density should not exceed 1.0×10^6 cells/mL. Cells can be propagated up to two months after thawing but not in excess of 30 passages post thawing.

At least two weeks after thawing, the cells will undergo a reactivity check. Only the cells which pass the reactivity check will be used in subsequent studies. Routine cell culture activities and reactivity check assay will be documented in the cell culture records and briefly summarized in the study report.

Prior to an assay, cells will be seeded in culture flasks at densities of 0.1 to 0.2×10^6 cells/mL and pre-cultured for approximately 72 or 48 hours, respectively. The culture conditions and cell density defined for this pre-assay culture conditioning should be maintained as consistently as possible to ensure optimal CD54 and CD86 induction and expression. On the day of testing, cells will be harvested from the culture flasks and seeded into 24-well plates, as described in section 7.4.3 for the dose range finding assay, or section 7.5.3 for the definitive assays.

7.4 Dose Range Finding Assay

A dose range finding assay will be conducted to determine the doses to be used in the definitive assays. The highest dose in the definitive assays will be selected by the Study Director which may be 1.2-fold higher than the calculated CV75 concentration (i.e., the test article concentration resulting in 75% cell viability relative to the solvent control).

7.4.1 Solvent Selection

A solubility test may be performed prior to the dose range finding assay in order to determine the most appropriate solvent. The evaluation of solvents should start with saline (0.9% NaCl) or cell culture medium, followed by DMSO. Other solvents may be attempted, and if used, must not adversely affect cell viability in the assays. If other solvents are used, solvent controls will be tested concurrently with the test article dilutions in the assays. Solubility is required for this assay. If solubility cannot be achieved, the Sponsor will be contacted regarding how to proceed. Test articles which do not form solutions (e.g., are noted as cloudy or form precipitates) may be sonicated and/or heated at 37±1°C in an attempt to further

solubilize the test article. In some cases, or under the guidance of the Sponsor, the sonication and/or heating may affect the stability of the test article and therefore heating and sonication would not be used for test article preparation. Solubility of the test articles should be evaluated at a maximum concentration of 100 mg/mL in saline or 500 mg/mL in DMSO or another appropriate solvent.

NOTE: The OECD Test Guideline specifies that in the absence of cytotoxicity in an initial dose range finding assay, test articles prepared in saline to a stock concentration of 100 mg/mL may be retested using a higher stock concentration up to a maximum of 500 mg/mL. Accordingly, at the Study Director's discretion, the dose range finding assay may be conducted using a maximum saline stock concentration of up to 500 mg/mL.

7.4.2 Preparation of Dilutions

The test and control articles will be prepared on the day of testing and applied to the test system within one hour of preparation to minimize potential for chemical degradation or breakdown. Based upon the results of the solubility test, the test articles will be dissolved to the maximum appropriate concentration determined in the solubility test, or up to a maximum final concentration of 100 mg/mL in saline (or up to 500 mg/mL in saline; see NOTE in section 7.4.1), or to a maximum final concentration of 500 mg/mL in DMSO. Other concentrations and solvents can be used if determined appropriate by the Study Director and/or Sponsor.

From the initial test article dilution, 2-fold serial dilutions will be prepared using the same solvent to obtain eight serial stock dilutions. These stock dilutions will then be further diluted 50-fold (for test articles diluted in saline) or 250-fold (for test articles diluted in DMSO) in the culture medium (2X dosing dilutions). These dosing dilutions are prepared at 2X the desired final concentration so that when 500 µL of each dosing dilution are added to 500 µL of cell suspension in the 24-well plate, a 1X final dose concentration is achieved.

The solvent control will be culture medium for test articles diluted in saline, or DMSO in culture medium for test articles diluted in DMSO. A single concentration of the solvent control(s) will be prepared in culture medium and dosed on the cells in the same manner as the test article(s) so that the final concentration of DMSO on the cells is 0.2%.

The positive control will be DNCB prepared at a stock concentration of 2 mg/mL in DMSO. The working solution of DNCB will be prepared by making an 8 µg/mL dilution of the stock in culture medium. The working solution of DNCB will be dosed on the cells in the same manner as the test article(s).

7.4.3 Preparation of the Test System

On the day of dosing, cells will be collected by centrifugation (200-300g, in a centrifuge set to 5 minutes at room temperature). The cells will be resuspended in

fresh culture medium to a density of 2.0×10^6 cells/mL, and 500 µL of the cell suspension will be seeded into the appropriate wells of a 24-well plate (resulting in 1.0×10^6 cells/well). The plates will be maintained at standard culture conditions.

7.4.4 Test System Exposure

The 2X dosing dilutions will be applied to the cells by pipetting 500 µL of each of the 2X dosing dilutions directly to the appropriate wells containing 500 µL of cell suspension. The treated plates will be sealed with plate sealers prior to incubation (to avoid evaporation or cross-contamination of volatile test articles), and will be incubated for 24±0.5 hours at standard culture conditions with at least 1 agitation per each day.

7.4.5 Cytotoxicity Assessment - Propidium Iodide (PI) Staining

After 24±0.5 hours of exposure, the samples will be removed from the 24-well plates and added to labeled micro-centrifuge tubes. The cells will be collected by centrifugation (200-300g, in a centrifuge set for 5 minutes and 4°C). The supernatants will be carefully decanted into a waste container. The remaining cell pellets will be resuspended with 1 mL of FACS buffer and centrifuged again using the above centrifuge settings and decanting the supernatant. The rinsing process is performed 2 additional times using 1 mL of FACS buffer.

After the three rinses, each cell pellet will be resuspended in 600 µL of FACS buffer and 200 µL of the suspension will be transferred to the appropriate wells of a 96-well round-bottom plate. Propidium Iodide will be added to the appropriate samples of the 96-well plate to make a final concentration of 0.625 µg/mL of PI in the plate.

7.4.6 Cytotoxicity Measurement and Calculation of CV75

The PI uptake will be analyzed using flow cytometry. Cells stained with PI represent the non-viable cell population and will be gated out to identify the viable populations. Approximately 10,000 living (PI negative) cells will be acquired. When the cell viability is low, up to approximately 30,000 cells including dead cells can be acquired. Alternatively, the data acquisition can be finished one minute after the initiation. The cell viability will be calculated (e.g. PI negative events versus total events).

The CV75 value, a concentration expected to result in 75% cell viability, will be calculated using the following formula:

$$\text{Log of CV75} = \frac{(75\text{-}C)\text{Log}(B) - (75\text{-}A)\text{Log}(D)}{A\text{-}C}$$

Where:
A is the minimum concentration with cell viability over 75%
C is the maximum concentration with cell viability below 75%
B and D are the viabilities associated with A and C, respectively

The CV75 value will be used to calculate the test article concentrations tested in the definitive assays. The range of doses used in the definitive assays may be modified at the Study Director's discretion.

The dose range finding assay may be repeated if the results of the dose range finding assay aren't sufficient to select doses for the definitive assays.

7.5 Definitive Assay

7.5.1 Test Article Dose Selection

Seven serial doses using a typical dilution factor of 1.2 to 1.5 will be prepared such that eight doses will be tested in the definitive assay. If there was insufficient cytotoxicity in the dose finding assay (i.e. CV75 > highest prepared dose), the highest soluble concentration of test article, up to a maximum stock concentration of 500 mg/mL in either saline or DMSO may be selected. At the Study Director's discretion and justification, the range of doses and the dilution factor to be used in the definitive assay may be modified.

7.5.2 Preparation of Stock and 2X Dosing Dilutions

The same solvent used in the dose range finding assay will be used to dissolve the test article in the definitive assays. The test article will be prepared as stock concentrations corresponding to 100-fold (for saline) or 500-fold (for DMSO). Seven serial dilutions using a dilution factor of 1.2-1.5 will be made using the same solvent to obtain eight serial dilutions. These dilutions will then be further diluted 50-fold (for test articles diluted in saline) or 250-fold (for test articles diluted in DMSO) in the culture medium (2X dosing dilutions). These dosing dilutions are prepared at 2X the desired final concentration so that when 500 µL of each dosing dilution are added to 500 µL of cell suspension in the 24-well plate, a 1X final dose concentration is achieved. The test article dilutions should be exposed to the cells within one hour of preparation.

The solvent controls and the positive controls will be prepared in the same manner as for the dose range finding assay (section 7.4.2).

7.5.3 Preparation of the Test System

On the day of dosing, the cells to be used in the assay will be prepared in the same manner as for the dose range finding assay (section 7.4.3).

7.5.4 Test System Exposure

The 2X dosing solutions will be applied to the cells by pipetting 500 µL of each of the 2X dosing solutions directly into the appropriate wells containing 500 µL of cell suspension. The treated plates will be sealed with plate sealers prior to incubation (to avoid evaporation or cross-contamination of volatile test article), and will be incubated for 24±0.5 hours at standard culture conditions with at least 1 agitation per each day.

For each test article, two independent trials with agreeing results are needed to make a prediction. In the case of incongruent results and/or at the Study Director's discretion, a third run (or more) may be completed.

7.5.5 Staining and Analysis

After 24±0.5 hours of exposure, the samples will be placed into labeled micro-centrifuge tubes and the cells will be collected by centrifugation as described in section 7.4.5. The supernatants will be carefully decanted into a waste container. The remaining cell pellets will be resuspended with 1 mL of FACS buffer and centrifuged. The rinsing process is performed 2 additional times using 1 mL of FACS buffer. Finally, cells will be resuspended in 600 µL of 0.01% (w/v) blocking suspension (prepared in FACS buffer from a 1% (w/v) stock suspension immediately before use) and incubated at 2-8°C for 15±1 minutes.

After the blocking step, the samples will be divided into 3 aliquots of 180 µL each into the designated wells of a 96-well round-bottom plate. The cells will be collected by centrifugation as described in section 7.4.5 and the supernatants will be aspirated without disturbing the cell pellet. A master mixture of each antibody (CD54, CD86 and mouse IgG isotype control) will be prepared based on the number of samples needing to be stained with each antibody so that each sample receives 50 µL of the appropriate antibody dose. For each test article dilution or control there will be three cell populations each treated with a different antibody mixture. There will be a separate cell population treated with FITC anti-CD54, FITC anti-CD86, and FITC isotype control. The antibody mixtures will be prepared in FACS buffer using the following ratios:

3 µL of CD54 to 50 µL total
6 µL of CD86 to 50 µL total
3 µL of isotype control to 50 µL total
Fifty microliters of each antibody mixture will be added to the appropriate wells of the 96-well plate. The plate will be gently agitated by hand to mix the reagents and then incubated in the dark at 2-8°C for 30±1 minutes. Following incubation, 150 µL of FACS buffer will be added to each well and the plate will be centrifuged as described in section 7.4.5. The wash step is repeated twice with 200 µL of FACS buffer. Finally, cells will be resuspended in 200 µL of FACS buffer. PI will be added to the appropriate wells of the 96-well plate to make a final concentration of 0.625 µg/mL of PI in the plate.

The expression of CD54, CD86, isotype control and PI uptake will be analyzed using flow cytometry. Cells stained with PI will be gated out to identify the viable populations. Approximately 10,000 living (PI negative) cells will be acquired. When the cell viability is low, up to 30,000 cells including dead cells can be acquired. Alternatively, the data acquisition can be finished one minute after the initiation. The cell viability will be calculated (e.g. PI negative events versus total events). In addition the MFI of the antibody stained cell populations will be calculated. The MFI values will be used to calculate the RFI values to determine skin sensitization predictions.

7.5.6 Data Analysis

The following plots are prepared using the flow cytometry software (MACSQuantify™ Version 2.10 / MACSQuant® Analyzer used for operation and data collection and FlowLogic 7.2.1 for data analysis):

- Side Scatter (SSC) versus Forward Scatter (FSC)
  FSC is a measure of cell size. SSC is a measure of cell granularity. This plot is created to confirm a single population of cells is present without excessive debris.

-2 Histogram Plots (Cell Count versus PI) (Cell Count versus FITC)
  These plots are used to determine the percentage of each cell population expressing PI (for cell viability) or FITC (for upregulation of CD54 and CD86).

A gate will be visually placed halfway between the peak of the PI negative fraction and the PI positive fraction on the histogram using the DNCB-treated isotype control cells. The PI negative fraction corresponds to living cells which are used for subsequent analysis. The MFI of the living populations of each cell sample is determined by the software and used in the following formula to determine the RFI values for each test article treated sample.

$$RFI = \frac{\text{MFI of test article treated cells} - \text{MFI of test article treated isotype control cells}}{\text{MFI of solvent treated control cells} - \text{MFI of solvent treated isotype control cells}}$$

The isotype controls consist of the same test article concentrations tested for the CD54 and CD86 staining, but these samples will be treated with isotype control consisting of mouse IgG. Use of the isotype control will allow for the distinction between specific CD54 and CD86 antibody binding and non-specific background antibody binding.

7.5.7 Prediction Model

Each test article will be tested in at least two independent definitive assays to derive a single prediction (skin sensitizer or non-sensitizer). The definitive assays may be performed on the same day provided that for each assay: a) independently

harvested cells will be used (i.e. cells collected from different culture flasks), and b) independent fresh stock solutions of the 2X dosing dilutions of the test articles and antibodies will be prepared.

If the RFI of CD86 is equal to or greater than 150 at any tested dose with >50% cell viability in at least two independent assays and/or if the RFI of CD54 is equal to or greater than 200 at any tested dose with >50% cell viability in at least two independent assays, the prediction will be considered as positive (sensitizer). Otherwise, the prediction will be considered as negative. In case the first two independent assays are not concordant, a third assay will be performed and typically the final prediction will be based on the mode of the conclusions from the three individual runs (i.e. 2 out of 3).

Test articles with limited solubility may still be tested at lower soluble concentrations or as suspensions. In such a case, a negative result will be considered inconclusive, whereas a positive result will be used to support the identification of the test article as a skin sensitizer.

For test articles considered to be sensitizers, two effective concentrations (EC) values, the EC150 for CD86 and EC200 for CD54 will be calculated using the following formulas. Two consecutive concentrations starting from the lowest dose and with RFI values greater than and less than 200 or 150 respectively, will be used in the EC calculations. The EC values represent the calculated test article concentration at which an RFI of 150 or 200 is achieved.

$$EC150\ (\text{for CD86}) = B_{dose} + [(150-B_{RFI})/(A_{RFI} - B_{RFI})(A_{dose}-B_{dose})]$$

$$EC200\ (\text{for CD54}) = B_{dose} + [(200-B_{RFI})/(A_{RFI} - B_{RFI})(A_{dose}-B_{dose})]$$

Where:
A_{dose} is the lowest concentration in µg/mL with RFI ≥150 (CD86) or 200 (CD54)
B_{dose} is the highest concentration in µg/mL with RFI <150 (CD86) or 200 (CD54)
A_{RFI} is the RFI value associated with A_{dose}
B_{RFI} is the RFI value associated with B_{dose}

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

The assay will be accepted if all of the following acceptance criteria are met:

8.1 The cell viability values of the solvent control(s) are > 90%.

8.2 For the solvent control(s), RFI values of both CD86 and CD54 are less than the positive criteria (CD86 RFI <150 and CD54 RFI <200).

8.3 For the positive control (DNCB), RFI values of both CD86 and CD54 are predicted to be positive (CD86 RFI ≥150 and CD54 RFI ≥200), and cell viability is >50%.

8.4 For the medium and solvent controls, the MFI ratio of both CD86 and CD54 to isotype control should be >105%.

8.5 The cell viability of the test article-treated cultures should be >50% in at least four doses.

9.0 EVALUATION OF TEST RESULTS

Negative results are acceptable only for test articles exhibiting cell viability <90% at the highest dose tested. Negative results with cell viabilities of ≥90% at the highest dose tested are not valid, and may require retesting at higher doses, unless the highest allowable doses were tested (i.e., up to 5000 µg/mL in saline, 1000 µg/mL in DMSO, or the highest soluble concentration).

10.0 REPORT

A report of the results of this study will be prepared by the Testing Laboratory and will accurately describe all methods used for generation and analysis of the data. A copy of the protocol used for the study, any amendments and any significant deviations from the protocol will appear as a part of the final report.

11.0 RECORDS AND ARCHIVES

A separate working notebook will be used to record the materials and procedures used to perform this study. Upon completion of the final report, all raw data, reports and specimens will be retained in the archives for a period of either a) 5 years, b) the length of time specified in the contract terms and conditions, or c) as long as the quality of the preparation affords evaluation, whichever is applicable.

12.0 TEST MATERIAL RETENTION

Unless indicated otherwise, all test articles provided by the sponsor will be retained for one year after completion of the final report. These test articles may be disposed after this 1 year retention period according to IIVS SOP. Unless indicated otherwise, dose solutions used for testing or analysis before or during the course of the assay will be discarded after testing.

13.0 PROTOCOL AMENDMENTS

When it becomes necessary to change the approved protocol for a specific study, the change and the reason for it should be put in writing and signed by the Study Director as soon as practical. When the change may impact the study design and/or execution, verbal agreement to make this change should be made between the Study Director and Sponsor. This document is then provided to the Sponsor and is attached to the protocol as an amendment.

14.0 REFERENCES

Ashikaga, T., *et al.* (2006) Development of an in vitro skin sensitization test using human cell lines: The human Cell Line Activation Test (h-CLAT) I. Optimization of the h-CLAT protocol. *Toxicol. In Vitro* 33 20:767–773.

DB-ALM (INVITTOX) Protocol 158: human Cell Line Activation Test (h-CLAT).

OECD (2018) *In vitro* skin sensitisation assays addressing the key event on activation of dendritic cells on the adverse outcome pathway for skin sensitisation 442E.

15.0 APPROVAL

[redacted]

4/17/2019
SPONSOR REPRESENTATIVE DATE

Judy Strickland, Ph.D., DABT
(Print or Type Name)

[redacted] 23 April 2019
IIVS STUDY DIRECTOR DATE

PROTOCOL ATTACHMENT 1

IIVS Test Article Designation	Sponsor Designation	Sponsor Designated Synonym
18AO64	ACTICIDE OIT	OIT
19AA05	2-Butyl-1,2-benzothiazolin-3-one (BBIT). Trade name: Vanquish 100	BBIT
19AA12	Mergal MITZ	CMIT/MIT Mixture
19AA13	Mergal BIT Technical	BIT
19AA98	KORDEK™ 573F BIOCIDE	MIT
19AB24	KATHON 287T industrial Microbicide	DCOIT

REGULATORY REQUIREMENTS:

Will this study be conducted according to **GLPs**? ☒ YES or ☐ NO

If **YES**, please indicate which agency(ies) guidelines are to be followed:
☐ OECD; ☐ FDA; ☐ Other:
☐ EPA TSCA (40 CFR part 792); ☒ EPA FIFRA (40 CFR part 160)

IIVS Study No.: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000
IIVS Project No.: 10426

PROTOCOL AMENDMENT I

SPONSOR:	National Institute of Environmental Health Sciences (NIEHS) NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
IIVS STUDY NO.:	18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

AMENDMENT(S):

1) Location: §4.0 TESTING FACILITY AND KEY PERSONNEL
§4.3 Study Director

Amendment: Replace "Greg Mun, B.A." with "Rishil J. Kathawala, Ph.D."

Reason: Rishil J. Kathawala is assuming the Study Director responsibility.

APPROVAL: [redacted] 20 May 2019

ASSU[redacted]ECTOR DATE

APPROVAL: [redacted] 5/20/19

TESTING FACILITY MANAGEMENT DATE

IIVS Study No.: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000
IIVS Project No.: 10426

PROTOCOL AMENDMENT II

SPONSOR:	National Institute of Environmental Health Sciences (NIEHS) NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
IIVS STUDY NO.:	18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

AMENDMENT(S):

1) Location: §2.0 SPONSOR
§2.3 Representative

§15.0 APPROVAL
SPONSOR REPRESENTATIVE

Amendment: Replace "Judy Strickland, Ph.D., DABT"

with "Judy Strickland, Ph.D., DABT
Integrated Laboratory Systems, Inc.,
Contractor supporting the NICEATM"

Reason: sponsor request

APPROVAL: [redacted] 9 October 2019

STUDY DIRECTOR DATE

IIVS Study No.: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000
IIVS Project No.: 10426

PROTOCOL AMENDMENT III

SPONSOR:	National Institute of Environmental Health Sciences (NIEHS) NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
IIVS STUDY NO.:	18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

AMENDMENT(S):

1) Location: PROTOCOL ATTACHMENT 1, Sponsor Designation in the table

Amendment:

Replace

19AA05	2-Butyl-1,2-benzothiazolin-3-one (BBIT). Trade name: Vanquish 100	BBIT

with

19AA05	2-Butyl-1,2-benzisothiazolin-3-one (BBIT). Trade name: Vanquish 100	BBIT

Reason: sponsor update

2) Location: PROTOCOL AMENDMENT II

Amendment: Add

Protocol page 1 and 12

To

1) Location:

Reason: protocol amendment II generation error

APPROVAL: [redacted] 28 October 2019

STUDY DIRECTOR DATE

APPENDIX B (Analyzed Data)

h-CLAT Dose Range Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Data Folder Name	Dose Range 052119
Plate Seeding Date	5/20/2019
Collection Date	5/21/2019
Cell Thaw Date	4/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	5/16/2019

TA ID	Well ID	Well Name	Living #Events	Living %Parent	Concentrations on the cells (µg/mL)						New Dose Range on the Cells (ug/mL)	New Dose Range in Primary Solvent (mg/mL)	New Dose Range in Media (µg/mL)
Controls	D1	Media	9997	98.21									
	C1	DMSO	9997	97.90									
	B1	DNCB	27088	68.09									
18AO64	A2	TA1D1	8164	11.75	1000	Solvent	DMSO				9.6	4.8	19.2
	B2	TA1D2	9810	75.61	500			Above Concentration*	7.8	Viability 77.18	8.0	4.0	16.0
	C2	TA1D3	6397	9.91	250			Below Concentration*	15.6	Viability 13.32	6.7	3.3	13.3
	D2	TA1D4	4194	6.69	125			Log CV75	0.903		5.6	2.8	11.1
	E2	TA1D5	2884	5.62	62.5			CV75	8.0	ug/mL on the cells	4.6	2.3	9.3
	F2	TA1D6	3437	7.24	31.3						3.9	1.9	7.7
	G2	TA1D7	7284	13.32	15.6						3.2	1.6	6.4
	H2	TA1D8	9992	77.18	7.81						2.7	1.3	5.4
19AA12	A3	TA2D1	6811	12.86	1000	Solvent	DMSO				38.2	19.1	76
	B3	TA2D2	5914	11.15	500			Above Concentration*	31.3	Viability 76.37	31.8	15.9	64
	C3	TA2D3	5971	10.61	250			Below Concentration*	62.5	Viability 25.87	26.5	13.3	53
	D3	TA2D4	5007	9.18	125			Log CV75	1.503		22.1	11.1	44
	E3	TA2D5	9943	25.87	62.5			CV75	31.8	ug/mL on the cells	18.4	9.2	37
	F3	TA2D6	9975	76.37	31.3						15.4	7.7	31
	G3	TA2D7	9995	96.92	15.6						12.8	6.4	26
	H3	TA2D8	9996	97.79	7.81						10.7	5.3	21
19AA13	A4	TA3D1	8970	13.25	1000	Solvent	DMSO				21.4	10.7	43
	B4	TA3D2	6564	10.12	500			Above Concentration*	15.6	Viability 85.07	17.8	8.9	36
	C4	TA3D3	5588	9.34	250			Below Concentration*	31.3	Viability 31.46	14.8	7.4	30
	D4	TA3D4	4028	7.36	125			Log CV75	1.250		12.4	6.2	25
	E4	TA3D5	3325	7.05	62.5			CV75	17.8	ug/mL on the cells	10.3	5.1	21
	F4	TA3D6	9965	31.46	31.3						8.6	4.3	17
	G4	TA3D7	9992	85.07	15.6						7.2	3.6	14
	H4	TA3D8	10000	94.48	7.81						6.0	3.0	12

New top stock concentration for the cytotoxicity assays

* - Refers to the concentration above or below 75% cell viability

Note: Cell dot plot showed dead events for this concentration. PI stained histogram peak appeared to have shifted to the left and fell under the living events gate when observing on flow cytometer. CB 5/22/19

h-CLAT Dose Range Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000; 19AB83.177000; 19AD20.177000

Data Folder Name	Dose Range 061319
Plate Seeding Date	6/12/2019
Collection Date	6/13/2019
Cell Thaw Date	4/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	5/16/2019

TA ID	Well ID	Well Name	Living #Events	Living %Parent	Concentrations on the cells (ug/mL)							New Dose Range on the Cells (ug/mL)	New Dose Range in Primary Solvent (mg/mL)	New Dose Range in Media (ug/mL)
Controls	D1	Media	10000	97.91										
	C1	DMSO	10000	97.77										
	B1	DNCB	22861	51.69										
19AA05	A2	TA1D1	9706	14.13	500	Solvent	DMSO					5.8	2.9	11.5
	B2	TA1D2	5072	21.03	250			Above Concentration*	3.9	Viability	82.07	4.8	2.4	9.6
	C2	TA1D3	10002	30.81	125			Below Concentration*	7.8	Viability	58.55	4.0	2.0	8.0
	D2	TA1D4	10002	34.81	62.5			Log CV75	0.682			3.3	1.7	6.7
	E2	TA1D5	3010	5.86	31.3			CV75	4.8	ug/mL on the cells		2.8	1.4	5.6
	F2	TA1D6	10005	23.06	15.6							2.3	1.2	4.6
	G2	TA1D7	10002	58.55	7.81							1.9	1.0	3.9
	H2	TA1D8	10000	82.07	3.91							1.6	0.8	3.2
19AA98	A3	TA2D1	6210	11.18	500	Solvent	DMSO					45	22.4	90
	B3	TA2D2	5558	10.44	250			Above Concentration*	31.3	Viability	82.02	37	18.7	75
	C3	TA2D3	5376	10.60	125			Below Concentration*	62.5	Viability	54.61	31	15.6	62
	D3	TA2D4	10002	54.61	62.5			Log CV75	1.572			26	13.0	52
	E3	TA2D5	10000	82.02	31.3			CV75	37.3	ug/mL on the cells		22	10.8	43
	F3	TA2D6	10000	96.01	15.6							18	9.0	36
	G3	TA2D7	10000	97.31	7.81							15	7.5	30
	H3	TA2D8	10000	97.77	3.91							12	6.2	25
19AB24	A4	TA3D1	7331	13.68	500	Solvent	DMSO							
	B4	TA3D2	8923	13.33	250			Above Concentration*	NA	Viability	NA			
	C4	TA3D3	8411	12.95	125			Below Concentration*	3.91	Viability	12.91			
	D4	TA3D4	7622	10.68	62.5			Log CV75	NA					
	E4	TA3D5	3405	6.04	31.3			CV75	<3.91	ug/mL on the cells				
	F4	TA3D6	3614	5.98	15.6									
	G4	TA3D7	2762	4.70	7.81									
	H4	TA3D8	7753	12.91	3.91									
19AB83	A5	TA4D1	10000	77.62	1000	Solvent	DMSO					1000	500	2000
	B5	TA4D2	10000	96.70	500			Above Concentration*	NA	Viability	NA	833	417	1667
	C5	TA4D3	10000	95.90	250			Below Concentration*	NA	Viability	NA	694	347	1389
	D5	TA4D4	10001	97.49	125			Log CV75	NA			579	289	1157
	E5	TA4D5	10001	97.63	62.5			CV75	NA	ug/mL on the cells		482	241	965
	F5	TA4D6	10000	97.30	31.3							402	201	804
	G5	TA4D7	10000	97.19	15.6							335	167	670
	H5	TA4D8	10000	97.68	7.81							279	140	558
19AD20	A6	TA5D1	6540	10.08	1000	Solvent	DMSO					11.5	5.7	22.9
	B6	TA5D2	7527	11.08	500			Above Concentration*	7.81	Viability	95.72	9.6	4.8	19.1
	C6	TA5D3	5749	9.87	250			Below Concentration*	15.63	Viability	24.47	8.0	4.0	15.9
	D6	TA5D4	6562	9.97	125			Log CV75	0.980			6.6	3.3	13.3
	E6	TA5D5	5810	9.45	62.5			CV75	9.6	ug/mL on the cells		5.5	2.8	11.1
	F6	TA5D6	5331	8.22	31.3							4.6	2.3	9.2
	G6	TA5D7	10015	24.47	15.6							3.8	1.9	7.7
	H6	TA5D8	10000	95.72	7.81							3.2	1.6	6.4
													New top stock concentration for the definitive assays	

* - Refers to the concentration above or below 75% cell viability

h-CLAT Dose Range Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Data Folder Name	Dose Range 062519
Plate Seeding Date	6/24/2019
Collection Date	6/25/2019
Cell Thaw Date	4/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	5/16/2019

TA ID	Well ID	Well Name	Living #Events	Living %Parent	Concentrations on the cells (ug/mL)	New Dose Range on the Cells (ug/mL)	New Dose Range in Primary Solvent (mg/mL)	New Dose Range in Media (ug/mL)
Controls	D1	Media	9997	98.11				
	C1	DMSO	9998	98.13				
	B1	DNCB	19526	67.98				
19AB24	A2	TA1D1	9816	14.92	3.00	1.37	0.69	2.74
	B2	TA1D2	9938	61.57	1.50	1.14	0.57	2.28
	C2	TA1D3	9991	95.70	0.75	0.95	0.48	1.90
	D2	TA1D4	9991	96.43	0.38	0.79	0.40	1.59
	E2	TA1D5	9992	96.36	0.19	0.66	0.33	1.32
	F2	TA1D6	9994	97.03	0.09	0.55	0.28	1.10
	G2	TA1D7	9995	96.50	0.05	0.46	0.23	0.92
	H2	TA1D8	9996	96.99	0.02	0.38	0.19	0.76

Solvent	DMSO		
Above Concentration*	0.75	Viability	95.70
Below Concentration*	1.50	Viability	61.57
Log CV75	0.058		
CV75	1.1	ug/mL on the cells	

New top stock concentration for the definitive assays

* - Refers to the concentration above or below 75% cell viability

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Plate Name	Definitive 061119
Plate Seeding Date	6/10/2019
Collection Date	6/11/2019
Cell Thaw Date	4/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	5/16/2019

Well ID	Well Name	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
A2	Media CD54	10001	9865	96.73	7.91
B2	Media CD86	10006	9987	96.33	22.70
C2	Media Isotype	10003	9881	96.23	6.16
E1	DMSO CD54	10004	9906	97.26	8.17
F1	DMSO CD86	10004	9982	97.25	22.49
G1	DMSO Isotype	10003	9848	96.38	5.63
D1	DNCB CD54	10015	9912	77.20	23.19
C1	DNCB CD86	10007	9979	79.20	32.86
B1	DNCB Isotype	18430	18167	78.97	6.21

Acceptance Criteria for a Valid Assay

Cell viabilities for medium and solvent controls are > 90%

Control	Viability	Criteria Met?
Medium	96.23	Yes
DMSO	96.38	Yes

Solvent control RFI values are negative responses

Control	RFI	Criteria Met?
DMSO CD54	145.14	Yes
DMSO CD86	101.93	Yes

MFI ratio of CD54/86 to isotype control for medium and solvent controls are > 105%

Control	Ratio	Criteria Met?
Medium CD54	128.41	Yes
Medium CD86	368.51	Yes
DMSO CD54	145.12	Yes
DMSO CD86	399.47	Yes

DNCB RFI values are positive and cell viability is > 50%

Control	RFI	Criteria Met?
DNCB CD54	668.50	Yes
DNCB CD86	158.07	Yes

Control	Viability	Criteria Met?
DNCB	78.97	Yes

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Plate Name	Definitive 061119
Plate Seeding Date	6/10/2019
Collection Date	6/11/2019
Cell Thaw Date	4/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	5/16/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
18AO64	A4	TA1D1 CD54	9.6	10015	9748	76.97	18.14	455.91
	B4	TA1D2 CD54	8.0	10007	9805	90.69	15.61	338.58
	C4	TA1D3 CD54	6.7	10006	9882	87.77	15.96	345.67
	D4	TA1D4 CD54	5.6	10007	9906	88.43	17.62	426.77
	E4	TA1D5 CD54	4.6	10007	9904	85.02	19.62	516.93
	F4	TA1D6 CD54	3.9	10006	9897	88.19	19.55	529.92
	G4	TA1D7 CD54	3.2	10009	9913	86.90	17.65	460.63
	H4	TA1D8 CD54	2.7	6808	6704	83.96	13.76	321.26
	A5	TA1D1 CD86	9.6	10013	9953	78.13	23.70	101.66
	B5	TA1D2 CD86	8.0	10012	9961	89.50	22.01	88.97
	C5	TA1D3 CD86	6.7	10003	9970	87.96	29.29	131.14
	D5	TA1D4 CD86	5.6	10008	9989	90.46	28.32	127.76
	E5	TA1D5 CD86	4.6	10008	9981	84.12	24.41	106.29
	F5	TA1D6 CD86	3.9	10009	9985	90.03	25.57	115.54
	G5	TA1D7 CD86	3.2	10006	9976	88.69	23.16	102.08
	H5	TA1D8 CD86	2.7	10010	9976	84.61	23.09	103.74
	A6	TA1D1 Isotype Control	9.6	10012	9729	80.33	6.56	
	B6	TA1D2 Isotype Control	8.0	10007	9825	89.87	7.01	
	C6	TA1D3 Isotype Control	6.7	10010	9884	87.52	7.18	
	D6	TA1D4 Isotype Control	5.6	10005	9886	89.55	6.78	
	E6	TA1D5 Isotype Control	4.6	10008	9857	84.94	6.49	
	F6	TA1D6 Isotype Control	3.9	10006	9828	89.67	6.09	
	G6	TA1D7 Isotype Control	3.2	10010	9844	88.40	5.95	
	H6	TA1D8 Isotype Control	2.7	10005	9774	85.06	5.60	

Highest Concentration below 200	NA	RFI	NA
Lowest Concentration above 200	2.7	RFI	321.26
EC200	<2.7		
Is the TA a sensitizer?	Yes		

Highest Concentration below 150	9.6	RFI	101.66
Lowest Concentration above 150	NA	RFI	NA
EC150	>9.6		
Is the TA a sensitizer?	No		

s viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Solvent DMSO

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	10004	9906	97.26	8.17
CD86	F1	10004	9982	97.25	22.49
Isotype	G1	10003	9848	96.38	5.63

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Plate Name	Definitive 061119
Plate Seeding Date	6/10/2019
Collection Date	6/11/2019
Cell Thaw Date	4/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	5/16/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
19AA12	A7	TA2D1 CD54	38.2	3727	3692	26.07	8.74	64.57
	B7	TA2D2 CD54	31.8	6320	6268	26.53	8.19	60.63
	C7	TA2D3 CD54	26.5	6794	6747	23.28	8.42	98.03
	D7	TA2D4 CD54	22.1	10036	9918	54.66	10.91	198.03
	E7	TA2D5 CD54	18.4	10014	9873	77.25	8.93	157.87
	F7	TA2D6 CD54	15.4	10007	9831	90.27	7.87	129.53
	G7	TA2D7 CD54	12.8	10003	9847	95.02	8.36	120.08
	H7	TA2D8 CD54	10.7	10002	9800	96.26	7.39	111.02
	A8	TA2D1 CD86	38.2	4672	4638	22.22	21.37	84.64
	B8	TA2D2 CD86	31.8	6524	6496	24.71	19.46	75.98
	C8	TA2D3 CD86	26.5	6859	6844	23.59	22.45	97.98
	D8	TA2D4 CD86	22.1	10053	10036	53.53	21.89	94.96
	E8	TA2D5 CD86	18.4	10013	10000	75.56	19.27	85.11
	F8	TA2D6 CD86	15.4	10016	10003	89.20	17.26	75.21
	G8	TA2D7 CD86	12.8	10005	9968	95.09	19.86	86.30
	H8	TA2D8 CD86	10.7	10004	9966	95.95	15.14	62.69
	A9	TA2D1 Isotype Control	38.2	4538	4488	23.63	7.10	
	B9	TA2D2 Isotype Control	31.8	6635	6572	24.01	6.65	
	C9	TA2D3 Isotype Control	26.5	7128	7062	22.53	5.93	
	D9	TA2D4 Isotype Control	22.1	10048	9879	51.27	5.88	
	E9	TA2D5 Isotype Control	18.4	10020	9728	74.53	4.92	
	F9	TA2D6 Isotype Control	15.4	10012	9685	89.33	4.58	
	G9	TA2D7 Isotype Control	12.8	10010	9818	94.58	5.31	
	H9	TA2D8 Isotype Control	10.7	10005	9700	96.38	4.57	

Highest Concentration below 200	22.1	RFI	198.03
Lowest Concentration above 200	NA	RFI	NA
EC200	>22.1		
Is the TA a sensitizer?	No		
Highest Concentration below 150	22.1	RFI	94.96
Lowest Concentration above 150	NA	RFI	NA
EC150	>22.1		
Is the TA a sensitizer?	No		

s viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Solvent DMSO

Viability <50% CB 6/14/19

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	10004	9906	97.26	8.17
CD86	F1	10004	9982	97.25	22.49
Isotype	G1	10003	9848	96.38	5.63

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Plate Name	Definitive 061119
Plate Seeding Date	6/10/2019
Collection Date	6/11/2019
Cell Thaw Date	4/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	5/16/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
19AA13	A10	TA3D1 CD54	21.4	10028	9964	38.66	18.66	349.21
	B10	TA3D2 CD54	17.8	10035	9935	48.68	33.99	1035.04
	C10	TA3D3 CD54	14.9	10009	9899	75.45	27.86	816.14
	D10	TA3D4 CD54	12.4	10013	9871	87.15	20.03	542.91
	E10	TA3D5 CD54	10.3	10014	9871	90.19	18.24	456.30
	F10	TA3D6 CD54	8.6	10008	9859	94.35	13.91	333.07
	G10	TA3D7 CD54	7.2	10012	9810	95.42	12.28	264.96
	H10	TA3D8 CD54	6.0	10005	9770	96.55	10.48	198.03
	A11	TA3D1 CD86	21.4	10037	10012	38.02	22.88	77 64
	B11	TA3D2 CD86	17.8	10029	10004	52.88	26.47	111.33
	C11	TA3D3 CD86	14.9	10010	9980	75.78	30.00	135.65
	D11	TA3D4 CD86	12.4	10017	9982	89.05	29.52	138.08
	E11	TA3D5 CD86	10.3	10013	9972	90.17	25.64	112.63
	F11	TA3D6 CD86	8.6	10008	9977	94.92	23.71	108.30
	G11	TA3D7 CD86	7.2	10008	9955	95.85	23.34	105.52
	H11	TA3D8 CD86	6.0	10004	9912	96.57	21.12	92.94
	A12	TA3D1 Isotype Control	21.4	9060	9001	41.65	9.79	
	B12	TA3D2 Isotype Control	17.8	10029	9879	56.01	7.70	
	C12	TA3D3 Isotype Control	14.9	10012	9826	79.59	7.13	
	D12	TA3D4 Isotype Control	12.4	10011	9784	89.15	6.24	
	E12	TA3D5 Isotype Control	10.3	10014	9832	90.08	6.65	
	F12	TA3D6 Isotype Control	8.6	10009	9786	94.85	5.45	
	G12	TA3D7 Isotype Control	7.2	10003	9781	96.10	5.55	
	H12	TA3D8 Isotype Control	6.0	10001	9797	96.77	5.45	

Highest Concentration below 200	6.0	RFI	198.03
Lowest Concentration above 200	7.2	RFI	264.96
EC200	6.02		
Is the TA a sensitizer?	Yes		

Highest Concentration below 150	17.8	RFI	111.33
Lowest Concentration above 150	NA	RFI	NA
EC150	>17.8		
Is the TA a sensitizer?	No		

s viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Solvent DMSO

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	10004	9906	97.26	8.17
CD86	F1	10004	9982	97.25	22.49
Isotype	G1	10003	9848	96.38	5.63

Viability <50% CB 6/14/19

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Plate Name	Definitive 061819
Plate Seeding Date	6/17/2019
Collection Date	6/18/2019
Cell Thaw Date	4/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	5/16/2019

Well ID	Well Name	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
A2	Media CD54	9992	9827	98.38	7.66
B2	Media CD86	9992	9953	98.50	22.21
C2	Media Isotype	9996	9841	98.60	6.17
E1	DMSO CD54	9997	9831	98.50	7.36
F1	DMSO CD86	9999	9964	98.60	24.42
G1	DMSO Isotype	9994	9754	98.17	5.64
D1	DNCB CD54	9969	9787	71.02	31.26
C1	DNCB CD86	9970	9921	70.59	38.71
B1	DNCB Isotype	16393	15836	74.00	6.45

Acceptance Criteria for a Valid Assay

Cell viabilities for medium and solvent controls are > 90%

Control	Viability	Criteria Met?
Medium	98.60	Yes
DMSO	98.17	Yes

Solvent control RFI values are negative responses

Control	RFI	Criteria Met?
DMSO CD54	115.44	Yes
DMSO CD86	117.08	Yes

MFI ratio of CD54/86 to isotype control for medium and solvent controls are > 105%

Control	Ratio	Criteria Met?
Medium CD54	124.15	Yes
Medium CD86	359.97	Yes
DMSO CD54	130.50	Yes
DMSO CD86	432.98	Yes

DNCB RFI values are positive and cell viability is > 50%

Control	RFI	Criteria Met?
DNCB CD54	1442.44	Yes
DNCB CD86	171.78	Yes

Control	Viability	Criteria Met?
DNCB	74.00	Yes

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Plate Name	Definitive 061819
Plate Seeding Date	6/17/2019
Collection Date	6/18/2019
Cell Thaw Date	4/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	5/16/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
18AO64	A4	TA1D1 CD54	9.6	9968	9780	70.07	24.34	1104.65
	B4	TA1D2 CD54	8.0	9980	9778	87.25	16.54	631.40
	C4	TA1D3 CD54	6.7	9994	9847	86.57	19.25	790.70
	D4	TA1D4 CD54	5.6	9988	9880	84.98	20.85	854.07
	E4	TA1D5 CD54	4.6	9989	9885	82.51	29.44	1372.67
	F4	TA1D6 CD54	3.9	9990	9884	82.35	29.96	1421.51
	G4	TA1D7 CD54	3.2	9991	9881	82.33	27.04	1223.84
	H4	TA1D8 CD54	2.7	9993	9875	83.87	24.65	1109.30
	A5	TA1D1 CD86	9.6	9960	9926	69.60	22.27	90.15
	B5	TA1D2 CD86	8.0	9990	9961	88.10	24.53	100.37
	C5	TA1D3 CD86	6.7	9988	9959	84.59	22.40	89.19
	D5	TA1D4 CD86	5.6	9986	9967	83.43	25.46	102.77
	E5	TA1D5 CD86	4.6	9993	9961	80.63	22.79	90.31
	F5	TA1D6 CD86	3.9	9993	9952	81.14	21.06	82.80
	G5	TA1D7 CD86	3.2	9994	9960	81.80	22.04	85.46
	H5	TA1D8 CD86	2.7	9989	9953	80.37	21.21	83.28
	A6	TA1D1 Isotype Control	9.6	9965	9566	68.51	5.34	
	B6	TA1D2 Isotype Control	8.0	9984	9710	87.04	5.68	
	C6	TA1D3 Isotype Control	6.7	9991	9732	83.84	5.65	
	D6	TA1D4 Isotype Control	5.6	9984	9812	83.43	6.16	
	E6	TA1D5 Isotype Control	4.6	9993	9803	78.57	5.83	
	F6	TA1D6 Isotype Control	3.9	9990	9759	79.42	5.51	
	G6	TA1D7 Isotype Control	3.2	9990	9787	82.11	5.99	
	H6	TA1D8 Isotype Control	2.7	9996	9781	81.93	5.57	

Highest Concentration below 200	NA	RFI	NA
Lowest Concentration above 200	2.7	RFI	1109.30
EC200	<2.7		
Is the TA a sensitizer?	Yes		

Highest Concentration below 150	9.6	RFI	90.15
Lowest Concentration above 150	NA	RFI	NA
EC150	>9.6		
Is the TA a sensitizer?	No		

Is viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Solvent DMSO

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	9997	9831	98.50	7.36
CD86	F1	9999	9964	98.60	24.42
Isotype	G1	9994	9754	98.17	5.64

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Plate Name	Definitive 061819
Plate Seeding Date	6/17/2019
Collection Date	6/18/2019
Cell Thaw Date	4/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	5/16/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
19AA12	A7	TA2D1 CD54	38.2	5534	5455	19.54	5.55	42.44
	B7	TA2D2 CD54	31.8	7532	7474	19.50	7.65	82.56
	C7	TA2D3 CD54	26.5	9792	9683	25.31	8.29	226.16
	D7	TA2D4 CD54	22.1	9878	9750	38.94	11.69	377.33
	E7	TA2D5 CD54	18.4	9971	9689	73.90	8.20	240.70
	F7	TA2D6 CD54	15.4	9990	9737	86.92	7.11	158.14
	G7	TA2D7 CD54	12.8	9993	9703	95.39	6.11	95.35
	H7	TA2D8 CD54	10.7	9995	9775	96.79	6.25	79.07
	A8	TA2D1 CD86	38.2	5374	5351	19.21	17.18	65.81
	B8	TA2D2 CD86	31.8	6694	6675	19.11	22.00	83.97
	C8	TA2D3 CD86	26.5	9368	9347	25.36	20.01	83.12
	D8	TA2D4 CD86	22.1	9892	9870	37.62	24.92	105.01
	E8	TA2D5 CD86	18.4	9969	9947	73.72	16.86	68.16
	F8	TA2D6 CD86	15.4	9977	9963	87.42	16.63	65.18
	G8	TA2D7 CD86	12.8	9992	9970	95.56	17.52	69.49
	H8	TA2D8 CD86	10.7	9987	9964	97.06	18.35	71.67
	A9	TA2D1 Isotype Control	38.2	5462	5381	19.79	4.82	
	B9	TA2D2 Isotype Control	31.8	7184	7107	19.42	6.23	
	C9	TA2D3 Isotype Control	26.5	8712	8576	26.09	4.40	
	D9	TA2D4 Isotype Control	22.1	9873	9669	40.47	5.20	
	E9	TA2D5 Isotype Control	18.4	9958	9513	72.45	4.06	
	F9	TA2D6 Isotype Control	15.4	9979	9591	86.62	4.39	
	G9	TA2D7 Isotype Control	12.8	9993	9640	94.77	4.47	
	H9	TA2D8 Isotype Control	10.7	9993	9715	96.82	4.89	

Highest Concentration below 200	15.4	RFI	158.14
Lowest Concentration above 200	18.4	RFI	240.70
EC200	16.91		
Is the TA a sensitizer?	Yes		

Highest Concentration below 150	18.4	RFI	68.16
Lowest Concentration above 150	NA	RFI	NA
EC150	>18.4		
Is the TA a sensitizer?	No		

s viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Solvent DMSO

Viability <50% CB 6/24/19

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	9997	9831	98.50	7.36
CD86	F1	9999	9964	98.60	24.42
Isotype	G1	9994	9754	98.17	5.64

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Plate Name	Definitive 061819
Plate Seeding Date	6/17/2019
Collection Date	6/18/2019
Cell Thaw Date	4/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	5/16/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
19AA13	A10	TA3D1 CD54	21.4	9937	9889	38.42	14.17	278.49
	B10	TA3D2 CD54	17.8	9953	9842	47.68	32.05	1445.93
	C10	TA3D3 CD54	14.9	9963	9843	66.16	33.91	1655.81
	D10	TA3D4 CD54	12.4	9988	9884	80.33	29.55	1356.40
	E10	TA3D5 CD54	10.3	9987	9835	92.04	15.13	555.81
	F10	TA3D6 CD54	8.6	9993	9833	93.04	13.90	463.95
	G10	TA3D7 CD54	7.2	9990	9801	95.85	10.65	320.35
	H10	TA3D8 CD54	6.0	9995	9855	97.03	10.28	286.63
	A11	TA3D1 CD86	21.4	9930	9913	42.12	19.81	55.54
	B11	TA3D2 CD86	17.8	9945	9895	52.19	19.20	64.00
	C11	TA3D3 CD86	14.9	9967	9923	70.73	20.85	82.11
	D11	TA3D4 CD86	12.4	9990	9952	83.27	26.37	107.29
	E11	TA3D5 CD86	10.3	9986	9941	91.94	23.04	93.02
	F11	TA3D6 CD86	8.6	9987	9950	93.53	24.57	99.31
	G11	TA3D7 CD86	7.2	9997	9958	96.19	20.08	79.55
	H11	TA3D8 CD86	6.0	9997	9947	96.83	19.34	74.49
	A12	TA3D1 Isotype Control	21.4	9925	9831	39.05	9.38	
	B12	TA3D2 Isotype Control	17.8	9952	9692	46.33	7.18	
	C12	TA3D3 Isotype Control	14.9	9973	9657	68.48	5.43	
	D12	TA3D4 Isotype Control	12.4	9979	9748	79.40	6.22	
	E12	TA3D5 Isotype Control	10.3	9988	9749	91.57	5.57	
	F12	TA3D6 Isotype Control	8.6	9988	9783	93.92	5.92	
	G12	TA3D7 Isotype Control	7.2	9987	9742	95.96	5.14	
	H12	TA3D8 Isotype Control	6.0	9998	9819	97.29	5.35	

Highest Concentration below 200	NA	RFI	NA
Lowest Concentration above 200	6.0	RFI	286.63
EC200	<6.0		
Is the TA a sensitizer?	Yes		

Highest Concentration below 150	17.8	RFI	64.00
Lowest Concentration above 150	NA	RFI	NA
EC150	>17.8		
Is the TA a sensitizer?	No		

Is viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Solvent DMSO

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	9997	9831	98.50	7.36
CD86	F1	9999	9964	98.60	24.42
Isotype	G1	9994	9754	98.17	5.64

Viability <50% CB 6/24/19

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Plate Name	Definitive 070219
Plate Seeding Date	7/1/2019
Collection Date	7/2/2019
Cell Thaw Date	6/10/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	6/15/2019

Well ID	Well Name	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
A2	Media CD54	10000	9800	97.58	7.16
B2	Media CD86	10001	9923	98.12	18.74
C2	Media Isotype	10000	9801	98.15	5.15
E1	DMSO CD54	10000	9851	97.70	7.50
F1	DMSO CD86	10000	9964	97.93	19.71
G1	DMSO Isotype	10000	9791	98.29	4.91
D1	DNCB CD54	10000	9900	81.56	24.52
C1	DNCB CD86	10000	9963	81.25	28.47
B1	DNCB Isotype	18676	18317	83.31	5.05

Acceptance Criteria for a Valid Assay

Cell viabilities for medium and solvent controls are > 90%

Control	Viability	Criteria Met?
Medium	98.15	Yes
DMSO	98.29	Yes

Solvent control RFI values are negative responses

Control	RFI	Criteria Met?
DMSO CD54	128.86	Yes
DMSO CD86	108.90	Yes

MFI ratio of CD54/86 to isotype control for medium and solvent controls are > 105%

Control	Ratio	Criteria Met?
Medium CD54	139.03	Yes
Medium CD86	363.88	Yes
DMSO CD54	152.75	Yes
DMSO CD86	401.43	Yes

DNCB RFI values are positive and cell viability is > 50%

Control	RFI	Criteria Met?
DNCB CD54	751.74	Yes
DNCB CD86	158.24	Yes

Control	Viability	Criteria Met?
DNCB	83.31	Yes

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Plate Name	Definitive 070219
Plate Seeding Date	7/1/2019
Collection Date	7/2/2019
Cell Thaw Date	6/10/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	6/15/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
19AA12	A4	TA1D1 CD54	38.2	4958	4908	27.42	6.19	18.92
	B4	TA1D2 CD54	31.8	7756	7677	26.20	6.98	25.87
	C4	TA1D3 CD54	26.5	6663	6598	21.16	6.06	34.36
	D4	TA1D4 CD54	22.1	10003	9874	38.74	10.25	175.68
	E4	TA1D5 CD54	18.4	10000	9859	75.13	9.89	184.56
	F4	TA1D6 CD54	15.4	10001	9833	89.06	9.07	154.05
	G4	TA1D7 CD54	12.8	10000	9828	95.85	7.57	112.74
	H4	TA1D8 CD54	10.7	10000	9837	96.06	7.79	109.65
	A5	TA1D1 CD86	38.2	5237	5195	27.26	16.17	70.74
	B5	TA1D2 CD86	31.8	7046	7002	26.03	16.23	67.03
	C5	TA1D3 CD86	26.5	6809	6789	20.69	17.25	81.62
	D5	TA1D4 CD86	22.1	10002	9979	38.63	18.83	88.72
	E5	TA1D5 CD86	18.4	10002	9980	73.32	17.98	86.96
	F5	TA1D6 CD86	15.4	10000	9971	90.05	16.71	78.58
	G5	TA1D7 CD86	12.8	10000	9978	94.97	15.37	72.43
	H5	TA1D8 CD86	10.7	10001	9968	96.08	17.88	87.36
	A6	TA1D1 Isotype Control	38.2	4181	4125	25.43	5.70	
	B6	TA1D2 Isotype Control	31.8	7201	7128	25.25	6.31	
	C6	TA1D3 Isotype Control	26.5	6079	6010	20.80	5.17	
	D6	TA1D4 Isotype Control	22.1	10003	9878	38.06	5.70	
	E6	TA1D5 Isotype Control	18.4	10001	9838	74.04	5.11	
	F6	TA1D6 Isotype Control	15.4	10000	9821	90.03	5.08	
	G6	TA1D7 Isotype Control	12.8	10000	9793	95.79	4.65	
	H6	TA1D8 Isotype Control	10.7	10000	9818	96.86	4.95	

Highest Concentration below 200	18.4	RFI	184.56
Lowest Concentration above 200	NA	RFI	NA
EC200	>18.4		
Is the TA a sensitizer?	No		

Highest Concentration below 150	18.4	RFI	86.96
Lowest Concentration above 150	NA	RFI	NA
EC150	>18.4		
Is the TA a sensitizer?	No		

Is viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Viability < 50% MM 7/3/19

Solvent DMSO

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	10000	9851	97.70	7.50
CD86	F1	10000	9964	97.93	19.71
Isotype	G1	10000	9791	98.29	4.91

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Plate Name	Definitive 070219
Plate Seeding Date	7/1/2019
Collection Date	7/2/2019
Cell Thaw Date	6/10/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	6/15/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
19AA05	A7	TA2D1 CD54	5.8	10001	9932	80.90	38.80	1207.34
	B7	TA2D2 CD54	4.8	10002	9957	81.32	38.81	1180.69
	C7	TA2D3 CD54	4.0	10000	9954	84.65	28.79	797.30
	D7	TA2D4 CD54	3.3	10002	9959	91.83	25.50	693.44
	E7	TA2D5 CD54	2.8	10001	9931	88.55	19.22	468.73
	F7	TA2D6 CD54	2.3	10001	9932	94.19	16.60	383.40
	G7	TA2D7 CD54	1.9	10000	9903	94.01	13.82	299.23
	H7	TA2D8 CD54	1.6	10000	9915	96.20	12.11	240.15
	A8	TA2D1 CD86	5.8	10001	9988	82.77	30.12	152.64
	B8	TA2D2 CD86	4.8	10001	9983	79.27	29.07	140.81
	C8	TA2D3 CD86	4.0	10000	9993	86.45	33.25	169.66
	D8	TA2D4 CD86	3.3	10000	9987	89.47	34.27	180.61
	E8	TA2D5 CD86	2.8	10001	9985	88.96	33.08	175.68
	F8	TA2D6 CD86	2.3	10000	9979	93.32	30.79	162.97
	G8	TA2D7 CD86	1.9	10000	9977	94.71	25.83	133.51
	H8	TA2D8 CD86	1.6	10000	9977	95.11	23.43	118.51
	A9	TA2D1 Isotype Control	5.8	10000	9914	83.72	7.53	
	B9	TA2D2 Isotype Control	4.8	10001	9928	79.65	8.23	
	C9	TA2D3 Isotype Control	4.0	10003	9928	86.12	8.14	
	D9	TA2D4 Isotype Control	3.3	10000	9932	89.17	7.54	
	E9	TA2D5 Isotype Control	2.8	10000	9915	89.57	7.08	
	F9	TA2D6 Isotype Control	2.3	10000	9913	92.69	6.67	
	G9	TA2D7 Isotype Control	1.9	10000	9885	95.92	6.07	
	H9	TA2D8 Isotype Control	1.6	10000	9885	95.65	5.89	

Highest Concentration below 200	NA	RFI	NA
Lowest Concentration above 200	1.6	RFI	240.15
EC200	<1.6		
Is the TA a sensitizer?	Yes		

Highest Concentration below 150	1.9	RFI	133.51
Lowest Concentration above 150	2.3	RFI	162.97
EC150	2.12		
Is the TA a sensitizer?	Yes		

Is viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Solvent DMSO

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	10000	9851	97.70	7.50
CD86	F1	10000	9964	97.93	19.71
Isotype	G1	10000	9791	98.29	4.91

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AA98, AB24.177000

Plate Name	Definitive 070219
Plate Seeding Date	7/1/2019
Collection Date	7/2/2019
Cell Thaw Date	6/10/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	6/15/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
19AA98	A10	TA3D1 CD54	45	10000	9949	68.87	25.29	716.22
	B10	TA3D2 CD54	37	10000	9934	77.32	40.80	1337.45
	C10	TA3D3 CD54	31	10000	9919	89.90	20.34	555.60
	D10	TA3D4 CD54	26	10000	9910	94.01	17.82	469.11
	E10	TA3D5 CD54	22	10000	9863	95.10	14.85	380.69
	F10	TA3D6 CD54	18	10000	9871	95.96	15.75	409.27
	G10	TA3D7 CD54	15	10000	9875	95.92	15.83	413.90
	H10	TA3D8 CD54	12	10000	9858	97.37	12.83	310.42
	A11	TA3D1 CD86	45	10000	9978	69.32	20.06	90.00
	B11	TA3D2 CD86	37	10000	9972	78.98	29.22	155.81
	C11	TA3D3 CD86	31	10000	9967	90.98	24.33	124.19
	D11	TA3D4 CD86	26	10000	9980	92.87	23.62	121.28
	E11	TA3D5 CD86	22	10001	9972	96.38	18.56	91.69
	F11	TA3D6 CD86	18	10000	9954	96.61	20.68	104.93
	G11	TA3D7 CD86	15	10001	9955	97.59	18.61	91.22
	H11	TA3D8 CD86	12	10000	9950	97.43	16.85	81.49
	A12	TA3D1 Isotype Control	45	10000	9887	69.81	6.74	
	B12	TA3D2 Isotype Control	37	10001	9834	81.19	6.16	
	C12	TA3D3 Isotype Control	31	10000	9836	90.32	5.95	
	D12	TA3D4 Isotype Control	26	10000	9854	93.60	5.67	
	E12	TA3D5 Isotype Control	22	10000	9796	95.93	4.99	
	F12	TA3D6 Isotype Control	18	10000	9857	96.15	5.15	
	G12	TA3D7 Isotype Control	15	10000	9800	97.25	5.11	
	H12	TA3D8 Isotype Control	12	10000	9783	97.68	4.79	

Highest Concentration below 200	NA	RFI	NA
Lowest Concentration above 200	12	RFI	310.42
EC200	<12		
Is the TA a sensitizer?	Yes		
Highest Concentration below 150	31	RFI	124.19
Lowest Concentration above 150	37	RFI	155.81
EC150	35.87		
Is the TA a sensitizer?	Yes		

Is viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Solvent DMSO

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	10000	9851	97.70	7.50
CD86	F1	10000	9964	97.93	19.71
Isotype	G1	10000	9791	98.29	4.91

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AB98, AB24.177000; 19AB83.177000; 19AD20.177000

Plate Name	Definitive 071119
Plate Seeding Date	7/10/2019
Collection Date	7/11/2019
Cell Thaw Date	6/10/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	6/25/2019

Well ID	Well Name	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
A2	Media CD54	10002	9876	98.22	7.35
B2	Media CD86	10002	9970	98.70	25.74
C2	Media Isotype	10005	9839	98.57	6.10
E1	DMSO CD54	10001	9922	98.96	7.73
F1	DMSO CD86	10003	9990	98.80	22.22
G1	DMSO Isotype	10001	9870	98.69	5.69
D1	DNCB CD54	10013	9926	76.31	27.78
C1	DNCB CD86	10016	9995	76.30	39.01
B1	DNCB Isotype	16150	15879	78.22	6.34

Acceptance Criteria for a Valid Assay

Cell viabilities for medium and solvent controls are > 90%

Control	Viability	Criteria Met?
Medium	98.57	Yes
DMSO	98.69	Yes

Solvent control RFI values are negative responses

Control	RFI	Criteria Met?
DMSO CD54	163.20	Yes
DMSO CD86	84.16	Yes

MFI ratio of CD54/86 to isotype control for medium and solvent controls are > 105%

Control	Ratio	Criteria Met?
Medium CD54	120.49	Yes
Medium CD86	421.97	Yes
DMSO CD54	135.85	Yes
DMSO CD86	390.51	Yes

DNCB RFI values are positive and cell viability is > 50%

Control	RFI	Criteria Met?
DNCB CD54	1050.98	Yes
DNCB CD86	197.64	Yes

Control	Viability	Criteria Met?
DNCB	78.22	Yes

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AB98, AB24.177000; 19AB83.177000; 19AD20.177000

Plate Name	Definitive 071119
Plate Seeding Date	7/10/2019
Collection Date	7/11/2019
Cell Thaw Date	6/10/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	6/25/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
19AA05	A4	TA1D1 CD54	5.8	10017	9960	77.75	28.34	976.47
	B4	TA1D2 CD54	4.8	10014	9947	82.11	18.51	532.84
	C4	TA1D3 CD54	4.0	10013	9948	86.71	16.86	475.49
	D4	TA1D4 CD54	3.3	10003	9942	90.44	14.37	351.47
	E4	TA1D5 CD54	2.8	10006	9909	93.07	11.18	229.90
	F4	TA1D6 CD54	2.3	10002	9910	93.77	11.41	228.43
	G4	TA1D7 CD54	1.9	10002	9867	95.05	9.29	159.31
	H4	TA1D8 CD54	1.6	10005	9897	95.91	9.26	144.12
	A5	TA1D1 CD86	5.8	10015	9989	79.44	35.18	161.89
	B5	TA1D2 CD86	4.8	10015	10001	84.70	33.52	156.56
	C5	TA1D3 CD86	4.0	10005	9994	86.38	32.25	151.78
	D5	TA1D4 CD86	3.3	10011	9995	90.26	30.98	143.86
	E5	TA1D5 CD86	2.8	10009	9991	91.67	24.57	109.38
	F5	TA1D6 CD86	2.3	10008	9997	94.91	28.44	131.22
	G5	TA1D7 CD86	1.9	10004	9980	95.19	21.61	94.19
	H5	TA1D8 CD86	1.6	10006	9993	95.46	24.70	111.19
	A6	TA1D1 Isotype Control	5.8	10006	9902	79.34	8.42	
	B6	TA1D2 Isotype Control	4.8	10013	9934	83.21	7.64	
	C6	TA1D3 Isotype Control	4.0	10009	9918	87.58	7.16	
	D6	TA1D4 Isotype Control	3.3	10004	9934	90.50	7.20	
	E6	TA1D5 Isotype Control	2.8	10005	9891	93.18	6.49	
	F6	TA1D6 Isotype Control	2.3	10002	9924	94.75	6.75	
	G6	TA1D7 Isotype Control	1.9	10004	9842	95.54	6.04	
	H6	TA1D8 Isotype Control	1.6	10003	9907	96.43	6.32	

Highest Concentration below 200	1.9	RFI	159.31
Lowest Concentration above 200	2.3	RFI	228.43
EC200	2.13		
Is the TA a sensitizer?	Yes		

Highest Concentration below 150	3.3	RFI	143.86
Lowest Concentration above 150	4.0	RFI	151.78
EC150	3.86		
Is the TA a sensitizer?	Yes		

Is viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Solvent DMSO

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	10001	9922	98.96	7.73
CD86	F1	10003	9990	98.80	22.22
Isotype	G1	10001	9870	98.69	5.69

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AB98, AB24.177000; 19AB83.177000; 19AD20.177000

Plate Name	Definitive 071119
Plate Seeding Date	7/10/2019
Collection Date	7/11/2019
Cell Thaw Date	6/10/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	6/25/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
19AA98	A7	TA2D1 CD54	45	10016	9926	72.18	29.61	1175.00
	B7	TA2D2 CD54	37	10014	9873	88.37	21.83	770.10
	C7	TA2D3 CD54	31	10007	9821	91.42	16.41	542.65
	D7	TA2D4 CD54	26	10010	9810	94.40	13.79	404.90
	E7	TA2D5 CD54	22	10004	9774	94.88	10.96	296.57
	F7	TA2D6 CD54	18	10006	9834	95.86	11.88	299.51
	G7	TA2D7 CD54	15	10005	9708	97.11	7.76	162.75
	H7	TA2D8 CD54	12	10002	9801	97.76	9.21	183.82
	A8	TA2D1 CD86	45	10022	9995	71.79	22.52	102.12
	B8	TA2D2 CD86	37	10011	9989	89.74	27.48	129.22
	C8	TA2D3 CD86	31	10006	9983	92.39	22.78	105.51
	D8	TA2D4 CD86	26	10003	9982	94.56	23.58	109.20
	E8	TA2D5 CD86	22	10006	9981	95.05	20.50	94.31
	F8	TA2D6 CD86	18	10006	9970	96.06	20.66	90.08
	G8	TA2D7 CD86	15	10005	9966	97.38	16.68	74.05
	H8	TA2D8 CD86	12	10003	9967	97.71	18.14	76.71
	A9	TA2D1 Isotype Control	45	10015	9715	71.31	5.64	
	B9	TA2D2 Isotype Control	37	10012	9728	89.32	6.12	
	C9	TA2D3 Isotype Control	31	10010	9676	91.86	5.34	
	D9	TA2D4 Isotype Control	26	10003	9683	94.71	5.53	
	E9	TA2D5 Isotype Control	22	10004	9606	95.19	4.91	
	F9	TA2D6 Isotype Control	18	10006	9778	96.22	5.77	
	G9	TA2D7 Isotype Control	15	10002	9532	97.72	4.44	
	H9	TA2D8 Isotype Control	12	10003	9753	97.48	5.46	

Highest Concentration below 200	15	RFI	162.75
Lowest Concentration above 200	18	RFI	299.51
EC200	16.11		
Is the TA a sensitizer?	Yes		
Highest Concentration below 150	45	RFI	102.12
Lowest Concentration above 150	NA	RFI	NA
EC150	>45		
Is the TA a sensitizer?	No		

Is viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Solvent DMSO

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	10001	9922	98.96	7.73
CD86	F1	10003	9990	98.80	22.22
Isotype	G1	10001	9870	98.69	5.69

h-CLAT Definitive Assay

Study Number: 18AO64, 19AA05, AA12-AA13, AB98, AB24.177000; 19AB83.177000; 19AD20.177000

Plate Name	Definitive 071119
Plate Seeding Date	7/10/2019
Collection Date	7/11/2019
Cell Thaw Date	6/10/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	6/25/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
19AB24	A10	TA3D1 CD54	1.37	10065	9973	52.34	14.59	322.06
	B10	TA3D2 CD54	1.14	10021	9939	77.36	15.54	399.02
	C10	TA3D3 CD54	0.95	10016	9909	92.82	13.24	331.37
	D10	TA3D4 CD54	0.79	10012	9905	94.13	14.21	386.76
	E10	TA3D5 CD54	0.66	10009	9861	97.17	7.88	100.00
	F10	TA3D6 CD54	0.55	10002	9892	97.99	7.65	72.55
	G10	TA3D7 CD54	0.46	10001	9783	98.13	6.83	52.94
	H10	TA3D8 CD54	0.38	10002	9884	98.05	7.48	70.10
	A11	TA3D1 CD86	1.37	10068	10034	53.71	21.95	84.27
	B11	TA3D2 CD86	1.14	10018	9995	75.80	26.52	115.67
	C11	TA3D3 CD86	0.95	10007	9987	92.91	24.90	111.43
	D11	TA3D4 CD86	0.79	10005	9984	94.87	22.14	95.70
	E11	TA3D5 CD86	0.66	10003	9982	97.85	21.01	91.77
	F11	TA3D6 CD86	0.55	10006	9977	98.00	20.36	85.84
	G11	TA3D7 CD86	0.46	10001	9979	98.08	17.76	72.66
	H11	TA3D8 CD86	0.38	10002	9973	98.42	18.77	76.95
	A12	TA3D1 Isotype Control	1.37	10070	9920	48.85	8.02	
	B12	TA3D2 Isotype Control	1.14	10023	9870	75.19	7.40	
	C12	TA3D3 Isotype Control	0.95	10010	9862	93.16	6.48	
	D12	TA3D4 Isotype Control	0.79	10008	9850	95.00	6.32	
	E12	TA3D5 Isotype Control	0.66	10005	9848	98.06	5.84	
	F12	TA3D6 Isotype Control	0.55	10006	9856	98.56	6.17	
	G12	TA3D7 Isotype Control	0.46	10003	9825	98.54	5.75	
	H12	TA3D8 Isotype Control	0.38	10005	9873	98.66	6.05	

Highest Concentration below 200	0.66	RFI	100.00
Lowest Concentration above 200	0.79	RFI	386.76
EC200	0.71		
Is the TA a sensitizer?	Yes		

Highest Concentration below 150	1.37	RFI	84.27
Lowest Concentration above 150	NA	RFI	NA
EC150	>1.37		
Is the TA a sensitizer?	No		

s viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Solvent DMSO

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	10001	9922	98.96	7.73
CD86	F1	10003	9990	98.80	22.22
Isotype	G1	10001	9870	98.69	5.69

h-CLAT Definitive Assay

Study Number: 18O64, 19AA05, AA12-13, AA98, AB24.177000;19AB83.177000;19AD20.177000

Plate Name	Definitive 082019
Plate Seeding Date	8/19/2019
Collection Date	8/20/2019
Cell Thaw Date	7/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	8/13/2019

Well ID	Well Name	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
A2	Media CD54	9998	9909	98.59	7.54
B2	Media CD86	9997	9983	98.36	14.49
C2	Media Isotype	9997	9902	98.16	5.88
E1	DMSO CD54	9999	9858	98.69	6.97
F1	DMSO CD86	9999	9987	98.77	13.37
G1	DMSO Isotype	9992	9843	98.34	5.17
D1	DNCB CD54	9975	9910	82.09	31.29
D2	DNCB CD86	6127	6114	88.23	18.64
C1	DNCB Isotype	15262	15037	81.26	4.96

Acceptance Criteria for a Valid Assay

Cell viabilities for medium and solvent controls are > 90%

Control	Viability	Criteria Met?
Medium	98.16	Yes
DMSO	98.34	Yes

Solvent control RFI values are negative responses

Control	RFI	Criteria Met?
DMSO CD54	108.43	Yes
DMSO CD86	95.24	Yes

MFI ratio of CD54/86 to isotype control for medium and solvent controls are > 105%

Control	Ratio	Criteria Met?
Medium CD54	128.23	Yes
Medium CD86	246.43	Yes
DMSO CD54	134.82	Yes
DMSO CD86	258.61	Yes

DNCB RFI values are positive and cell viability is > 50%

Control	RFI	Criteria Met?
DNCB CD54	1462.78	Yes
DNCB CD86	166.83	Yes

Control	Viability	Criteria Met?
DNCB	81.26	Yes

h-CLAT Definitive Assay

Study Number: 18O64, 19AA05, AA12-13, AA98, AB24.177000;19AB83.177000;19AD20.177000

Plate Name	Definitive 082019
Plate Seeding Date	8/19/2019
Collection Date	8/20/2019
Cell Thaw Date	7/26/2019
Did cells pass the reactivity check?	Yes
Reactivity Date	8/13/2019

	Well ID	Well Name	Final Test Article Concentration (µg/mL)	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean	Calculated RFI
19AB24	A4	TA1D1 CD54	1.37	9978	9907	82.22	16.72	537.22
	B4	TA1D2 CD54	1.14	9994	9927	95.34	15.28	516.11
	C4	TA1D3 CD54	0.95	9992	9887	97.28	10.68	297.78
	D4	TA1D4 CD54	0.79	9996	9890	97.57	8.64	160.00
	E4	TA1D5 CD54	0.66	9997	9920	98.24	7.94	121.67
	F4	TA1D6 CD54	0.55	9997	9935	98.35	8.03	135.56
	G4	TA1D7 CD54	0.46	9995	9903	98.56	7.30	101.11
	H4	TA1D8 CD54	0.38	9996	9926	98.47	7.47	109.44
	A5	TA1D1 CD86	1.37	9963	9955	82.76	18.23	136.34
	B5	TA1D2 CD86	1.14	69660	69575	95.66	16.99	134.15
	C5	TA1D3 CD86	0.95	77687	77567	97.03	15.03	118.41
	D5	TA1D4 CD86	0.79	80181	80075	97.73	15.17	114.76
	E5	TA1D5 CD86	0.66	65317	65255	98.30	15.31	116.59
	F5	TA1D6 CD86	0.55	62205	62139	98.60	14.18	104.76
	G5	TA1D7 CD86	0.46	32514	32466	98.60	12.95	91.10
	H5	TA1D8 CD86	0.38	47173	47122	98.26	14.97	115.49
	A6	TA1D1 Isotype Control	1.37	38423	38079	78.44	7.05	
	B6	TA1D2 Isotype Control	1.14	71911	71147	94.41	5.99	
	C6	TA1D3 Isotype Control	0.95	76699	75592	96.71	5.32	
	D6	TA1D4 Isotype Control	0.79	78334	77488	97.45	5.76	
	E6	TA1D5 Isotype Control	0.66	62668	62170	97.93	5.75	
	F6	TA1D6 Isotype Control	0.55	74747	74106	98.28	5.59	
	G6	TA1D7 Isotype Control	0.46	60800	60085	98.23	5.48	
	H6	TA1D8 Isotype Control	0.38	51086	50651	98.16	5.50	

Highest Concentration below 200	0.79	RFI	160.00
Lowest Concentration above 200	0.95	RFI	297.78
EC200	0.84		
Is the TA a sensitizer?	Yes		

Highest Concentration below 150	1.4	RFI	136.34
Lowest Concentration above 150	NA	RFI	NA
EC150	>1.37		
Is the TA a sensitizer?	No		

s viability ≥ 50% for at least 4 concentrations?	Yes
Is viability of highest concentration < 90%?	Yes

Solvent DMSO

	Well ID	Viable Events	Positive Events	% Viable	Living FITC Geometric Mean
CD54	E1	9999	9858	98.69	6.97
CD86	F1	9999	9987	98.77	13.37
Isotype	G1	9992	9843	98.34	5.17

APPENDIX C (Certificates of Analysis)

SIGMA-ALDRICH

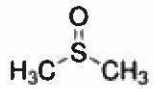
sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
Dimethyl sulfoxide - for HPLC, ≥99.7%

Product Number:	**34869**
Batch Number:	**SHBJ7917**
Brand:	SIGALD
CAS Number:	67-68-5
MDL Number:	MFCD00002089
Formula:	C2H6OS
Formula Weight:	78.13 g/mol
Quality Release Date:	09 JAN 2018
Expiration Date:	JUN 2021



Test	Specification	Result
Appearance (Color)	Colorless	Colorless
Appearance (Form)	Liquid	Liquid
UV Absorbance 350nm	≤ 0.01	< 0.01
UV Absorbance 300nm	≤ 0.10	0.07
UV Absorbance 280nm	≤ 0.30	0.18
UV Absorbance 270nm	≤ 0.70	0.38
Purity (GC)	≥ 99.70 %	99.98 %
Water (by Karl Fischer)	≤ 0.2 %	< 0.1 %
Residue on Evaporation	≤ 0.002 %	< 0.001 %
Expiration Date Period 1260 Days	----------	----------

[redacted]
Michael Grady, Manager
Quality Control
Sheboygan Falls, WI US

Certificate of Analysis

SIGMA-ALDRICH

Product Name	Sodium chloride solution, 0.9% in water, BioXtra, suitable for cell culture
Product Number	S8776
Product Brand	SIGMA
CAS Number	7647-14-5
Molecular Formula	NaCl
Molecular Weight	58.44

TEST	SPECIFICATION	LOT RNBH2274 RESULTS
Storage:		ROOM TEMPERATURE
Print Date:		21 DEC 2018
Date of QC Release:		21 DEC 2018
Place of Manufacture:		Irvine, United Kingdom
Production Date:		DEC 2018
Appearance (Turbidity)	Clear	Clear
Appearance (Colour)	Colorless	Colorless
Appearance (Form)	Solution	Solution
pH	-	7.0
Osmolality	278 - 308 mOs/kg	290 mOs/kg
Salt Toxicity Test	Pass	Pass
Cell Line	Cell Line - Cell Types	ED1
Key Element Conc - ICP (Sodium)	3.3 - 3.7 g/l	3.6 g/l
Sterility	Pass	Pass
Endotoxin Level	<= 1.0 EU/ml	< 1.0 EU/ml

[redacted]

Jane Findlay, Manager
Quality Control
Irvine, United Kingdom

SIGMA-ALDRICH

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	1-CHLORO-2,4-DINITROBENZENE
	>= 99 %
Product Number:	237329
Batch Number:	BCBS4201V
Brand:	Aldrich
CAS Number:	97-00-7
Formula:	$ClC_6H_3(NO_2)_2$
Formula Weight:	202.55
Quality Release Date:	04 JUL 2016

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	FAINT YELLOW TO YELLOW	FAINT YELLOW
APPEARANCE (FORM)	POWDER OR CRYSTALS	CRYSTALS
PURITY (GC AREA %)	≥ 99.0 %	99.0 %
INFRARED SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS

[redacted]

Dr. Claudia Geltner
Manager Quality Control
Buchs, Switzerland

SIGMA-ALDRICH
3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	1-CHLORO-2,4-DINITROBENZENE
	>= 99 %
Product Number:	237329
Batch Number:	BCBW5262
Brand:	Aldrich
CAS Number:	97-00-7
Formula:	$ClC_6H_3(NO_2)_2$
Formula Weight:	202.55
Quality Release Date:	07 FEB 2018

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	FAINT YELLOW TO YELLOW	YELLOW
APPEARANCE (FORM)	POWDER OR CRYSTALS	CRYSTALS
PURITY (GC AREA %)	≥ 99.0 %	99.8 %
INFRARED SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS

[redacted]

Dr. Reinhold Schwenninger
Quality Assurance
Buchs, Switzerland

Date 2019-06-07 (YYYY-MM-DD) Time 20:31:17 (Greenwich Mean Time) Page 1 of 1

DOW

DP SPECIALTY ELECTRONIC MATERIALS US, INC.

Certificate of Analysis

Product Number 00010406546
Product Name

KORDEK™ 573F Industrial Microbiocide

Delivery No. /000000

Shipping Units 1.000 KG

Shipment No.

Customer Information

Customer Name

Specification Number 000000226833

Batch Number	YY00H3A451
Expiration Date	2019-09-10 (YYYY-MM-DD)
Manufacturing Date	2017-03-10 (YYYY-MM-DD)
Quantity	1.000 KG

Test	Unit	Lower Limit	Upper Limit	Value
Appearance	-	-	-	**Pass**
A.I. (MIT)	%	50.0	52.0	50.8
pH		3.0	6.0	3.6

For inquiries please contact Customer Service or local sales

Date 2019-07-19 (YYYY-MM-DD) Time 08:55:37 (Greenwich Mean Time) Page 1 of 1

IM AND HAAS INTERNATIONAL TRADING SHANGHAI CO., LTD. A Subsidiary of The Dow Chemical Company	DOW CHEMICAL (SHANGHAI) COMPANY LIMITED D BLOCK, 1/F,185 TAI GU RD WAIGAOQIAO FREE TRADE ZONE 200131 SHANGHAI

Certificate of Analysis

Product Number	00010269161
Product Name	KATHON™ 287T Industrial Microbicide
Delivery No.	810808143 / 000010
Order Number	106838704
Shipping Units	120.000 KG
Date Shipped	2017-07-26 (YYYY-MM-DD)
Shipment No.	30174145

Customer Information

Customer Name	DOW CHEMICAL (SHANGHAI)
Customer PO number	sample20170710
Specification Number	000000142005

Batch Number	YY00H77338
Expiration Date	2020-01-07 (YYYY-MM-DD)
Manufacturing Date	2017-07-07 (YYYY-MM-DD)
Quantity	120.000 KG
Weight	120.000 KG

Test	Unit	Lower Limit	Upper Limit	Value
Appearance	-	-	-	**Pass**
Color, Gardner VCS		**0**	**4**	**2**
Water Content	**%**	**0.00**	**0.07**	**0.02**
A.I. (DCOIT)	**%**	**95.0**	**100.0**	**99.3**
Hydrochloric Acid	**%**	**0.00**	**0.10**	**< 0.00**

For inquiries please contact Customer Service or local sales

Date 2017-07-26 (YYYY-MM-DD) Time 08:55:37 (Greenwich Mean Time) Page 1 of 1

:OHM AND HAAS INTERNATIONAL TRADING
SHANGHAI CO., LTD.
A Subsidiary of The Dow Chemical Company

DOW CHEMICAL (SHANGHAI)
COMPANY LIMITED
D BLOCK, 1/F,185 TAI GU RD
WAIGAOQIAO FREE TRADE ZONE
200131 SHANGHAI

Certificate of Analysis

Product Number	00010269161
Product Name	
KATHON™ 287T Industrial Microbicide	
Delivery No.	810808143 / 000010
Order Number	106838704
Shipping Units	120.000 KG
Date Shipped	2017-07-26 (YYYY-MM-DD)
Shipment No.	30174145

Customer Information

Customer Name	DOW CHEMICAL (SHANGHAI)
Customer PO number	sample20170710
Specification Number	000000142005

Batch Number	YY00H77338
Expiration Date	2019-07-07 (YYYY-MM-DD)
Manufacturing Date	2017-07-07 (YYYY-MM-DD)
Quantity	120.000 KG
.et Weight	120.000 KG

Test	Unit	Lower Limit	Upper Limit	Value
Appearance	-	-	-	**Pass**
Color, Gardner VCS		0	4	2
Water Content	%	0.00	0.07	0.02
A.I. (DCOIT)	%	95.0	100.0	99.3
Hydrochloric Acid	%	0.00	0.10	< 0.00

For inquiries please contact Customer Service or local sales
® ™ Trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow

TROY

The Gold Standard for Performance

Troy Chemical Corporation Inc.

Date: Jan-8-2019
Customer Name:
Customer Order Number:
Customer Code:
Quantity & Weight
Remarks:

CERTIFICATE OF ANALYSIS

Product: **Mergal BIT Technical**

Lot: **YL201811073**

Characteristics	Specifications	Actual Lot Analysis
BIT, % Appearance	83.5 min Light Yellow or Off-White Powder	85.2 Pass

Date of Manufacture: Nov 2018
Expiration Date: Nov 2021

This Certificate is generated from a computerized system by the QC Manager. Authorized signature is not required.

TROY

The Gold Standard for Performance

Troy Chemical Corporation Inc.

Date: Jan-07-2019
Customer Name:
Customer Order Number:
Customer Code:
Quantity & Weight
Remarks: Expiration Date is Oct 07,2020

CERTIFICATE OF ANALYSIS

Product: MERGAL MITZ

Lot: SLJ0229

Characteristics	Specification	Actual Lot Analysis
Appearance	Colorless Liquid to Light Yellow Liquid	Colorless Liquid to Light Yellow Liquid
5CMIT, %	10.0 – 11.6	10.8
MIT, %	3.0 – 4.1	3.4
5CMIT + MIT, %	14.0 Min.	14.2
D-CMIT, %	0.1 Max.	0.0
Color, Gardner	5 Max.	0.7
Density @ 20C	1.25 – 1.33	1.31
pH	4 Max.	3

Date of Manufacture: Oct-2018

This Certificate is generated from a computerized system by the QC Manager. Authorized signature is not required.

LONZA GLP SERVICES
1200 BLUEGRASS LAKES PARKWAY
ALPHARETTA, GA 30004

Certificate of Analysis

Test or Reference Substance Name: Vanquish 100

Lot Number: 6445 **Expiration Date (mm/dd/yyyy):** 05/15/2019

Storage Conditions: room temperature

Compound	Assay	Analytical Technique
2-Butyl-1,2-benzisothiazolin-3-one (BBIT)	98.9 %	HPLC

Comments:

Identity confirmed by LC-MS

Master Log Number/Notebook Number and page(s): SN 383-17B10BBIT/552

Characterization of this test or reference substance was performed under EPA FIFRA Good Laboratory Practice Standards (40 CFR 160).

Study Director: [redacted] Date: 05/17/2017
Management: [redacted] Date: 05/17/2017
QA: [redacted] Date: 05/17/2017

Revised June 20, 2014

EXACT COPY OF RAW DATA
SIGNATURE: [redacted]
DATE: 05-20-2019

TSOP027 ATTACHMENT 1

LONZA GLP SERVICES
1200 BLUEGRASS LAKES PARKWAY
ALPHARETTA, GA 30004

Certificate of Analysis

Test or Reference Substance Name: Vanquish 100 EPA Reg. No. 1258-1249
CAS No.: 4299-07-4 **Lot Number:** 6445
Manufacturing Date: 12/8/2015
Test Date: 05/15/2019 **Expiration Date** 05/15/2021
Storage: Room temperature

Compound	Assay	Analytical Technique
2-butyl-1,2-benzisothiazolin-3-one (BBIT)	98.4	HPLC

Comments:
N/A

Master Log Number/Notebook Number and page(s): SN 439-19B10BBIT/609 pages 3,4 and 5

Characterization of this test or reference substance was performed under EPA FIFRA Good Laboratory Practice Standards (40 CFR 160).

Study Director: [redacted] Date: 05/20/2019
Management: [redacted] Date: 05/20/2019
QA: [redacted] Date: 5/20/2019
Revised August 06, 2018

Certificate of Analysis

THOR

Print Date:	July 31, 2018
Issue Date:	July 31, 2018
Product:	ACTICIDE® OIT
Batch No:	MX-183774-2006
Production Date:	06/2018
Expiry Date*:	30-Jun-2020
Minimum shelf-life:	*24 months*

Analyzed Property	Unit	Results	Specification	Method
Appearance		OK	Clear yellow to brown liquid	QK 118
OIT	%	98.13	95 - 100	QK 101
Water content	%	0.34	0 - 0.5	QK 107

*If stored in accordance with chapters 7 & 10 of the Safety Data Sheet.

Some products are able to be retested and the expiry date extended if results warrant. Please contact your Sales Rep or Thor Specialties, Inc., directly for additional information.

The information presented above is believed to be accurate. However, said information and products are offered without warranty or guarantee except as to the composition and purity stated herein since the ultimate conditions of use and the variability of the materials treated are beyond our control.

This lot was manufactured in Querétaro, Mexico. It does not meet the eligibility requirements for NAFTA certification.

US Agent:
THOR SPECIALTIES, INC.
50 Waterview Drive• Shelton, CT 06484• U.S.A.
Telephone: (203) 516-6980 • Fax: (203) 954-0005
Email: info@thorsp.com
Lynn P. Tordo, Regulatory/QA Manager,
ltordo@thorsp.com

Thor GmbH
D-67346 Speyer
GERMANY
Tel: 0049 6232 6360
Fax: 0049 6232 636111
email: info@thor.com

Thor Specialties (UK) Limited
Cheshire CW9 6GB
ENGLAND
Tel: 0044 1606 818800
Fax: 0044 1606 818801
email: info@thor.uk.com

Thor Químicos de México,
Querétaro CP 76700
MEXICO
Tel: 0052 448 2752200
Fax: 0052 448 2752209
email: thor.mexico@thor.com

Thor Especialidades, S.A.
Barcelona SPAIN
Tel: 0034 93 633 26 00
Fax: 0034 93 633 37 13
email: thor@thor-spain.com

This Quality Assurance document has been generated by computer and is valid without signature.