MANUAL

AlphaLISA® Human hTREM2 Detection Kit

Product number: AL3152

Research Use Only. Not for use in diagnostic procedures.

Product Information

Application: This kit is designed for the quantitative determination of human hTREM2 using a homogeneous no wash AlphaLISA assay.

Kit contents: The kit contains 5 components: AlphaLISA Acceptor beads coated with anti-hTREM2 Antibody, Streptavidin-coated Donor beads, Biotinylated anti-hTREM2 antibody, Lyophilized hTREM2 and 10X AlphaLISA Immunoassay Buffer.

Sensitivity: Lower Detection Limit (LDL): 4.3 pg/mL
Lower Limit of Quantification (LLOQ): 13.8 pg/mL
EC_{50}: 19.5 ng/mL

Dynamic Range: 4 – 100 000 pg/mL

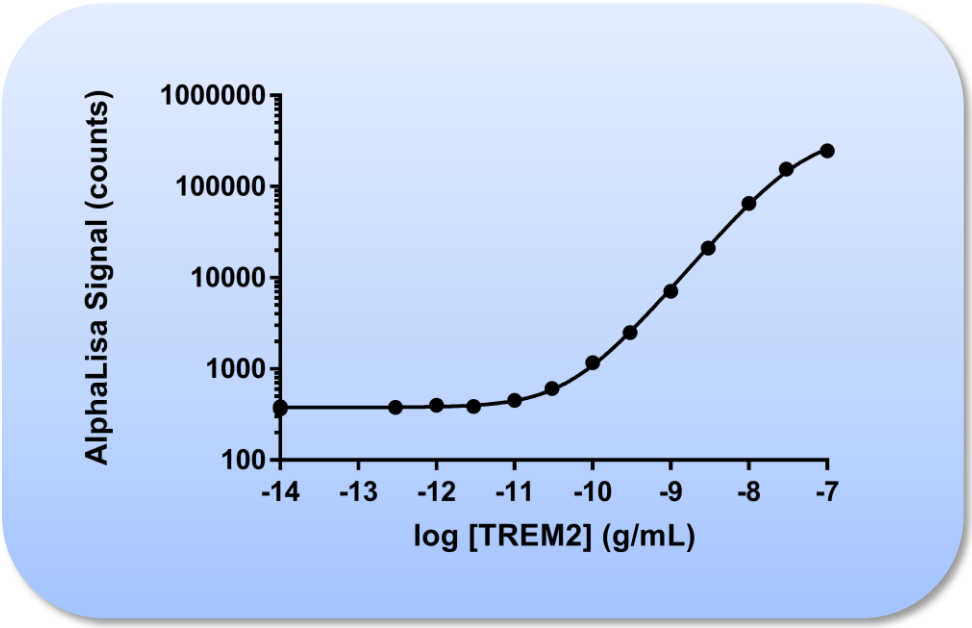


Figure 1. Typical sensitivity curve in AlphaLISA Immunoassay Buffer. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader 2102 with Alpha option.

Storage: Store kit in the dark at 4 ºC. For reconstituted analyte, aliquot and store at -20 ºC. Avoid freeze-thaw cycles.

Stability: This kit is stable for at least 6 months from the date of manufacture when stored in its original packaging and the recommended storage conditions.

Analyte of Interest

hTREM2 is a transmembrane molecule expressed on myeloid cells. It acts as the receptor for an unknown ligand to activate myeloid cells such as dendritic cells, increasing phagocytic activity. Recently, hTREM2 has been shown to be involved in neurodegenerative diseases such as ataxia, early dementia and Alzheimer's disease. Elevated levels of hTREM2 have been noted in human serum and cerebrospinal fluid as a response to Alzheimer's disease and are also used as markers for inflammation.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in human serum, human plasma and culture medias (DMEM and RPMI 1640) in a highly sensitive, quantitative, reproducible and user-friendly mode. In this AlphaLISA assay, a biotinylated anti-hTREM2 antibody binds to the streptavidin coated AlphaLISA Donor beads, while another anti-hTREM2 antibody is conjugated to AlphaLISA Acceptor beads. In the presence of hTREM2, the beads come into proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer within the Acceptor beads, resulting in emission with λ_{max} at 615 nm (Figure 2).

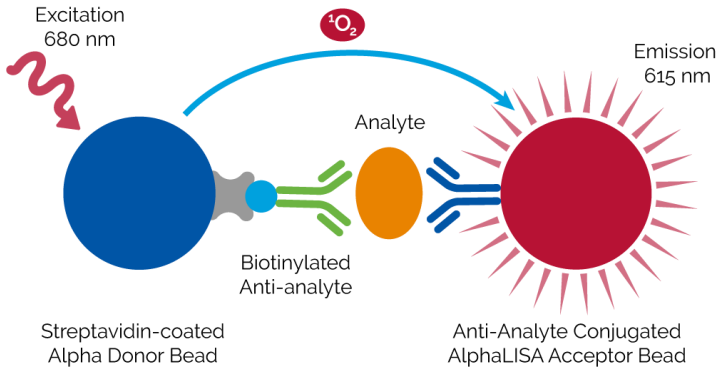


Figure 2. AlphaLISA human hTREM2 Detection Assay Principle.

Precautions

- The Alpha Donor beads are light sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- Take precautionary measures to avoid contamination of the reagent solutions.
- The biotinylated anti-hTREM2 antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Kit Content: Reagents and Materials

Kit components	AL3152HV 100 assay points***	AL3152C 500 assay points***	AL3152F 5000 assay points***
AlphaLISA Anti-hTREM2 Acceptor beads stored in PBS, 0.05% Kathon CG/ICP, pH 7.2	20 µL @ 5 mg/mL (1 brown tube, white cap)	50 µL @ 5 mg/mL (1 brown tube, white cap)	500 µL @ 5 mg/mL (1 brown tube, white cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon CG/ICP, pH 7.4	80 µL @ 5 mg/mL (1 brown tube, black cap)	200 µL @ 5 mg/mL (1 brown tube, black cap)	2 X 1 mL @ 5 mg/mL (2 brown tubes, black caps)
Biotinylated Anti-hTREM2 Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	20 µL @ 500 nM (1 tube, black cap)	50 µL @ 500 nM (1 tube, black cap)	500 µL @ 500 nM (1 tube, black cap)
Lyophilized hTREM2 Analyte*	0.1 µg (1 tube, clear cap)	0.1 µg (1 tube, clear cap)	0.1 µg (1 tube, clear cap)
AlphaLISA Immunoassay Buffer (10X) **	2 mL, 1 small bottle	10 mL, 1 medium bottle	100 mL, 1 large bottle

* Reconstitute lyophilized analyte in 100 µL Milli-Q® grade H_2O. The reconstituted analyte should be used within 60 minutes or aliquoted into screw-capped 0.5 mL polypropylene vials and stored at -20°C for future experiments. The aliquoted analyte at -20°C is stable up to 90 days. Avoid freeze-thaw cycles. One vial contains an amount of analyte sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # ALAL3152S).

** Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).

*** The number of assay points is based on an assay volume of 100 µL in 96-well plates or 50 µL in 384-well assay plates using the kit components at the recommended concentrations.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001% final in the assay) might decrease the AlphaLISA signal. Note that sodium azide from the biotinylated anti-hTREM2 antibody stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.	6050185
EnVision®-Alpha Reader	Revvity Inc.	-

Recommendations

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to pre-wet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000*g*, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O to dilute 10X AlphaLISA Immunoassay Buffer and to reconstitute the lyophilized analyte.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film in place.
- The AlphaLISA signal is detected with an EnVision Multilabel Plate Reader equipped with the Alpha option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve must be generated for each experiment.

Assay Procedure

- The manual described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The manuals also include testing samples in 452 wells. If different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly, as shown in the table below. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.
- The standard dilution manual is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.
- Use of four background points in triplicate (12 wells) is recommended when LDL/LLOQ is calculated. One background point in triplicate (3 wells) can be used when LDL/LLOQ is not calculated.

		Volume					
Format	**# of data points**	**Final**	**Sample**	**AlphaLISA Acceptor Beads**	**Biotinylated Antibody**	**SA-Donor beads**	**Plate recommendation**
AL3152HV	100	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL3152C	250	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290)
	500	50 µL	5 µL	10 µL	10 µL	25 µL	½ Area AlphaPlate-96 (cat # 6002350) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	2 µL	4 µL	4 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	10 µL	1 µL	2 µL	2 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL3152F	5 000	50 µL	5 µL	10 µL	10 µL	25 µL	½ Area AlphaPlate-96 (cat # 6002350) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	2 µL	4 µL	4 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	1 µL	2 µL	2 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

The following 3-Step manual described below is for 500 assay points including one standard curve (48 wells) and samples (452 wells). *If different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.*

1) Preparation of 1X AlphaLISA Immunoassay Buffer:
Add 1 mL of 10X AlphaLISA Immunoassay Buffer to 9 mL Milli-Q® grade H_2O.

2) Preparation of hTREM2 analyte standard dilutions:

   a. Reconstitute lyophilized hTREM2 (0.1 µg) in 100 µL Milli-Q® grade H_2O.
   b. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

Tube	Vol. of hTREM2 (µL)	Vol. of diluent (µL) *	[hTREM2] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of reconstituted hTREM2	90	1.00E-07	100 000
B	60 µL of tube A	140	3.00E-08	30 000
C	60 µL of tube B	120	1.00E-08	10 000
D	60 µL of tube C	140	3.00E-09	3 000
E	60 µL of tube D	120	1.00E-09	1 000
F	60 µL of tube E	140	3.00E-10	300
G	60 µL of tube F	120	1.00E-10	100
H	60 µL of tube G	140	3.00E-11	30
I	60 µL of tube H	120	1.00E-11	10
J	60 µL of tube I	140	3.00E-12	3
K	60 µL of tube J	120	1.00E-12	1
L	60 µL of tube K	140	3.00E-13	0.3
M ** (background)	0	100	0	0
N ** (background)	0	100	0	0
O ** (background)	0	100	0	0
P ** (background)	0	100	0	0

* Dilute standards in diluent (e.g. 1X AlphaLISA Immunoassay Buffer).
At low concentrations of analyte, a significant amount of analyte can bind to the vial. Therefore, load the analyte standard dilutions in the assay microplate within 60 minutes of preparation.

** Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

3) Preparation of 5X AlphaLISA Anti-hTREM2 Antibody Acceptor beads (50 µg/mL):

   a. Prepare just before use.
   b. Add 10 µL of 5 mg/mL AlphaLISA Anti-hTREM2 Antibody Acceptor to 990 µL of 1X AlphaLISA Immunoassay Buffer.

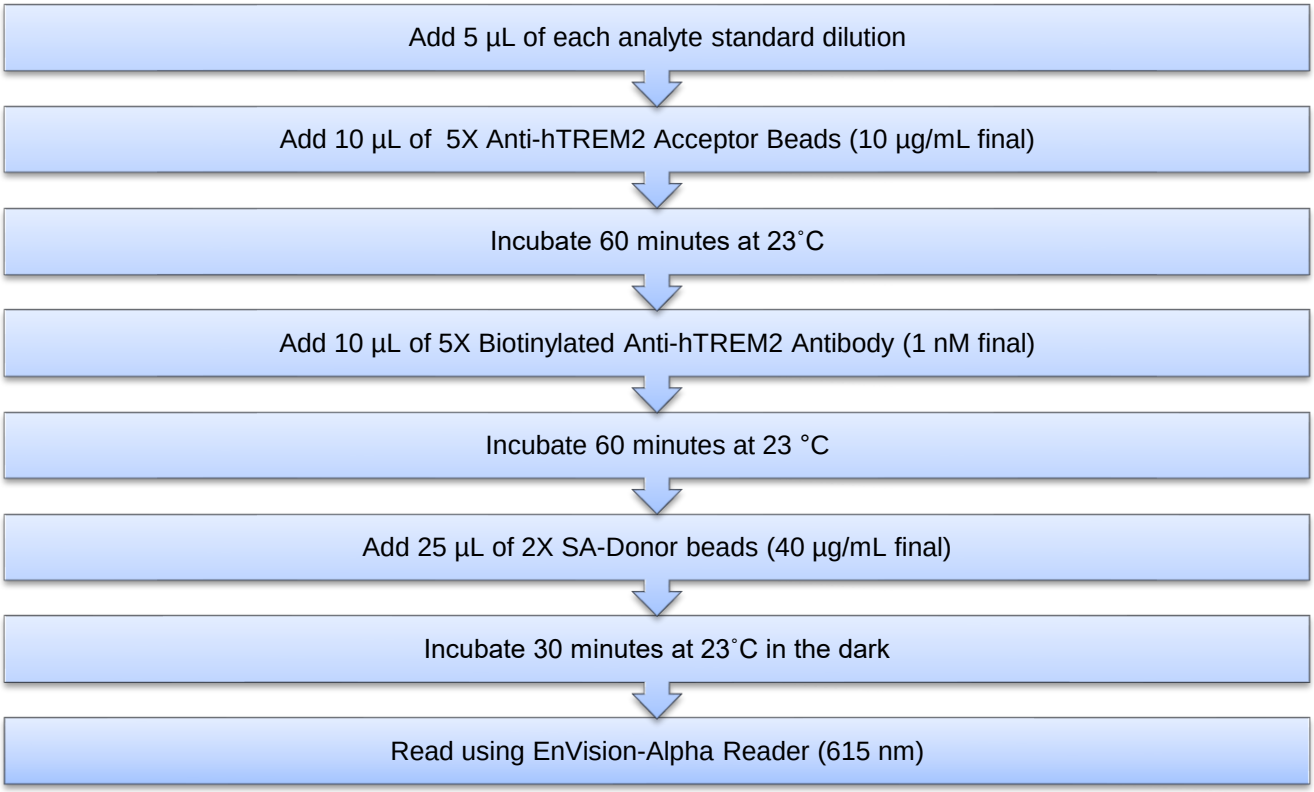
4) Preparation of 5X Biotinylated Anti-hTREM2 Antibody (5 nM):

   a. Prepare just before use.
   b. Add 10 µL of 500 nM Biotinylated Anti-hTREM2 Antibody to 990 µL of 1X AlphaLISA Immunoassay Buffer.

5) Preparation of 2X Streptavidin (SA) Donor beads (80 µg/mL):

   a. Prepare just before use.
   b. Keep the beads under subdued laboratory lighting.
   c. Add 32 µL of 5 mg/mL SA-Donor beads to 1968 µL of 1X AlphaLISA Immunoassay Buffer.

6) In a white OptiPlate (384 wells):



Data Analysis

- Calculate the average count value for the background wells.
- Generate a standard curve by plotting the AlphaLISA counts versus the concentration of analyte. A log scale can be used for either or both axes. No additional data transformation is required.
- Analyze data according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a $1/Y^2$ data weighting (the values at maximal concentrations of analyte after the hook point should be removed for correct analysis).
- The LDL is calculated by interpolating the average background counts (12 wells without analyte) + 3 x standard deviation value (average background counts + (3xSD)) on the standard curve.
- The LLOQ as measured here is calculated by interpolating the average background counts (12 wells without analyte) + 10 x standard deviation value (average background counts + (10xSD)) on the standard curve. Alternatively, the true LLOQ can be determined by spiking known concentrations of analyte in the matrix and measuring the percent recovery, and then determining the minimal amount of spiked analyte that can be quantified within a given limit (usually +/- 20% or 30% of the real concentration).
- Read from the standard curve the concentration of analyte contained in the samples.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Assay Performance Characteristics

AlphaLISA assay performance described below was determined to be a 3-step manual using AlphaLISA Immunoassay Buffer 1X as assay buffer. The analytes (standards) were prepared in Immunoassay Buffer 1X, DMEM + 10% FBS, RPMI 1640 + 10% FBS, 100% FBS and AlphaLISA Lysis Buffer 1X. All other components were prepared in AlphaLISA Immuno assay Buffer 1X.

- Assay Sensitivity:

The LDL was calculated as described above. The values correspond to the lowest concentration of analyte that can be detected in a volume of 5 µL sample using the recommended assay conditions.

LDL (pg/mL)	(Analyte diluent)	# of experiments
4.3	AlphaLISA Immunoassay buffer 1X	9
6.2	DMEM + 10% FBS	6
22.8	RPMI 1640 + 10% FBS	6
16.7	100% FBS	6
26.2	AlphaLISA Lysis buffer 1X	6

- Assay Precision:

The following assay precision data were calculated from the three independent assays using two different kit lots. In each lot, the analytes were prepared in Immunoassay Buffer 1X, DMEM + 10% FBS, RPMI 1640 + 10% FBS, 100% FBS and AlphaLISA Lysis Buffer 1X. All other components were prepared in AlphaLISA Immunoassay Buffer 1X. Each assay consisted of one standard curve comprising 12 data points (each in triplicate) and 12 background wells (no analytes). The assays were performed in 384-well plate format.

  - Intra-assay precision:

The intra-assay precision was determined using a total of 6 independent determinations in triplicate. Shown as CV%.

hTREM2	AlphaLISA Immunoassay Buffer 1X	DMEM + 10% FBS	RPMI 1640 + 10% FBS	100% FBS	AlphaLISA Lysis Buffer 1X
CV (%)	5.1	6.5	2.5	7.3	4.2

  - Inter-assay precision:

The inter-assay precision was determined using a total of 3 independent determinations with 9 measurements. Shown as CV%.

hTREM2	AlphaLISA Immunoassay Buffer 1X	DMEM + 10% FBS	RPMI 1640 + 10% FBS	100% FBS	AlphaLISA Lysis Buffer 1X
CV (%)	11.9	9.8	4.4	10.7	14.3

- Spike Recovery:

Three known concentrations of analyte were spiked into Immunoassay Buffer 1X, DMEM + 10% FBS, RPMI 1640 + 10% FBS, 100% FBS and AlphaLISA Lysis Buffer 1X. All samples, including non-spiked diluents were measured in the assay. Note that the analytes for the respective standard curves were prepared in Immunoassay Buffer 1X, DMEM + 10% FBS, RPMI 1640 + 10% FBS, 100% FBS and AlphaLISA Lysis Buffer 1X. All other assay components were diluted in AlphaLISA Immunoassay Buffer 1X.

Spiked hTREM2 (ng/mL)	% Recovery				
	AlphaLISA Immunoassay Buffer 1X	DMEM + 10% FBS	RPMI 1640 + 10% FBS	100% FBS	AlphaLISA Lysis Buffer 1X
10	114	101	89	116	101
1	105	93	91	86	101
0.1	124	99	99	108	92

- Specificity:

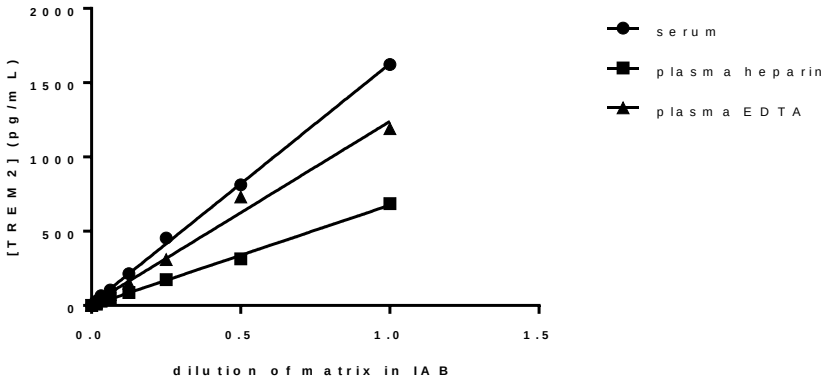
Cross-reactivity of the hTREM2 AlphaLISA Detection Kit was tested using the following proteins at 10 ng/mL in AlphaLISA Immunoassay Buffer 1X. The cross reactivities were calculated using the signals of 10 ng/mL hTREM2 as 100%.

Proteins	Cross Reactivity (%)
hTREM1	
mTREM2	<0.01
Bovine TREM2	0

- Human Serum Experiments:
    - Dilution Linearity

Normal human serum, normal human plasma (heparin treated) and normal human plasma (EDTA treated) samples were diluted with 100% FBS and the assay was performed along with a standard curve prepared in 100% FBS. Concentrations of hTREM2 in diluted samples were determined by interpolating to the standard curve. The other components (anti-hTREM2 acceptor beads, biotinylated anti-hTREM2 antibody, and SA-Donor beads) of the assays were prepared in AlphaLISA Immunoassay Buffer 1X. Excellent dilution linearity ($R^2 > 0.999$) was achieved in the human serum and plasma samples (2- to 64-fold dilution). The results are shown in the table and the figure below.

matrix Dilution Factor (x)	hTREM2 in human serum (pg/mL)	hTREM2 in human plasma (heparin treated) (pg/mL)	hTREM2 in human plasma (EDTA treated) (pg/mL)
1 (raw)	1622,1	685,5	1191,4
2	812,0	314,2	730,3
4	454,2	174,6	309,5
8	214,6	87,3	149,1
16	104,1	46,7	73,8
32	65,1	29,6	44,2
64	29,2	8,7	20,7



human serum R^2 = 0.999

human plasma heparin R^2 = 0.990

Human plasma EDTA R^2 = 0.989

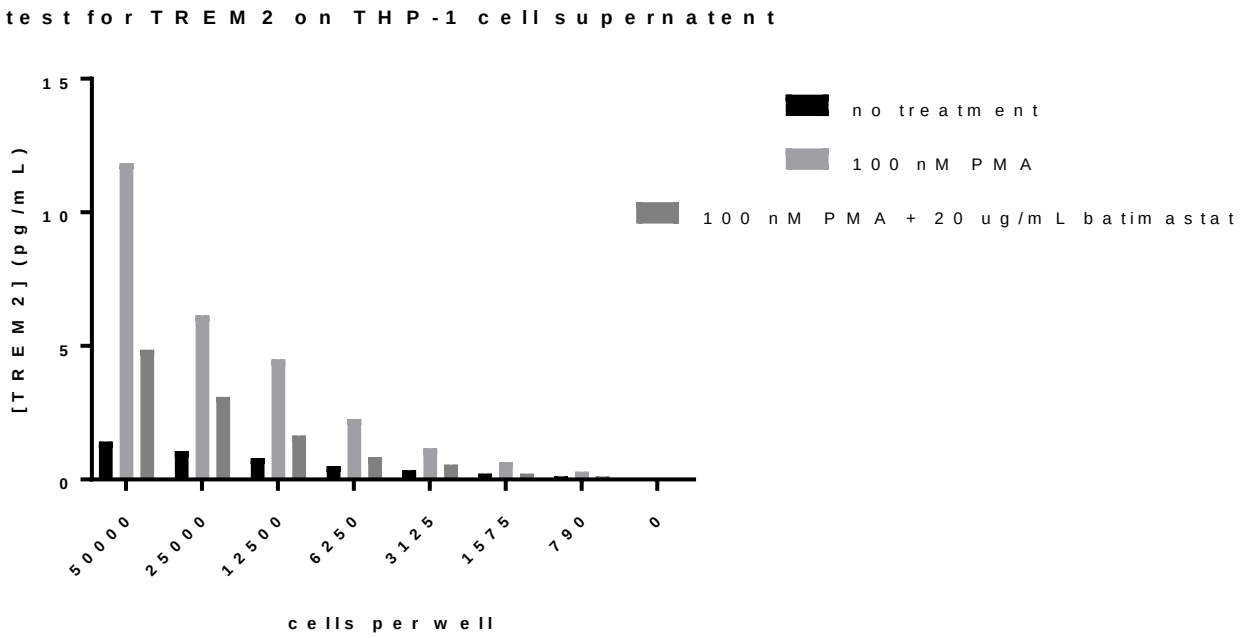
- Human Cells Experiments:

The human monocyte THP-1 was used for testing TREM2 secretion. THP-1 cells differentiated with phorbol-12-myristate-13-acetate (PMA) are known to secrete significant amounts of hTREM2. To confirm the results, inhibition of MMP proteases that are involved in TREM2 maturation and secretion were inhibited with the pan MMP inhibitor batimastat.

Cell growth and treatment:

1. A tube of frozen THP-1 cells was unfrozen and added to a T-75 flask with RPMI 1640 + 10% FBS.
2. Cells were grown until confluence (about 4 days).
3. Cells were harvested by centrifugation 5 minutes at 1200g.
4. Cells were washed twice with 20 mL cold sterile PBS.
5. Cells were resuspended in RPMI + 10% FBS and counted by the Trypan blue method.
6. Cells were plated in duplicate in the wells of a 96 well culture-treated plate.
7. Cells were plated first at 100 000 cells per well (1 000 000 cells per mL) in 100 uL
8. Cells were diluted in 1:2 sequential dilutions (10 dilutions) and a 0 cells control.
9. One series of cells was incubated alone.
10. One series of cells was incubated with 100 nM PMA
11. One series of cells was incubated with 100 nM PMA and 20 ug/mL batimastat.
12. The cells were incubated for 3 days at 37ºC with an atmosphere of 5% CO_2.

Supernatant of each well was tested in triplicate using the assay described in this document, with RPMI 1640 + 10% FBS used as the diluent for the standard curve.



Results show clear stimulation of secretion of hTREM2 upon differentiation. Levels of secreted TREM2 were lower when batimastat was added, but not eliminated, raising the possibility that proteases capable of maturation of TREM2 show redundancy.

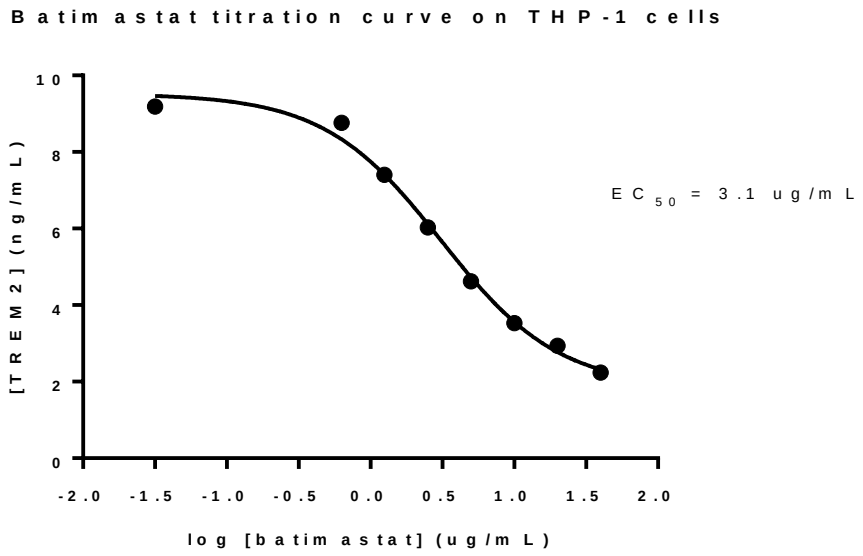
- Batimastat inhibition curve

Manual:

Cell growth and treatment:

1. A tube of frozen THP-1 cells was unfrozen and added to a T-75 flask with RPMI 1640 + 10% FBS.
2. Cells were grown until confluence (about 4 days).
3. Cells were harvested by centrifugation 5 minutes at 1200g.
4. Cells were washed twice with 20 mL cold sterile PBS.
5. Cells were resuspended in RPMI + 10% FBS and counted by the Trypan blue method.
6. Cells were plated in duplicate in the wells of a 96 well culture-treated plate.
7. Cells were plated at 100 000 cells per well (1 000 000 cells per mL) in 100 uL
8. Cells were incubated with 100 nM PMA and batimastat at 40, 20, 10, 5, 2.5, 1.25, 0.625 and 0 ug/mL
9. The cells were incubated for 3 days at 37ºC with an atmosphere of 5% CO_2.

Supernatant of each well was tested in triplicate using the assay described in this document, with RPMI 1640 + 10% FBS used as the diluent for the standard curve.

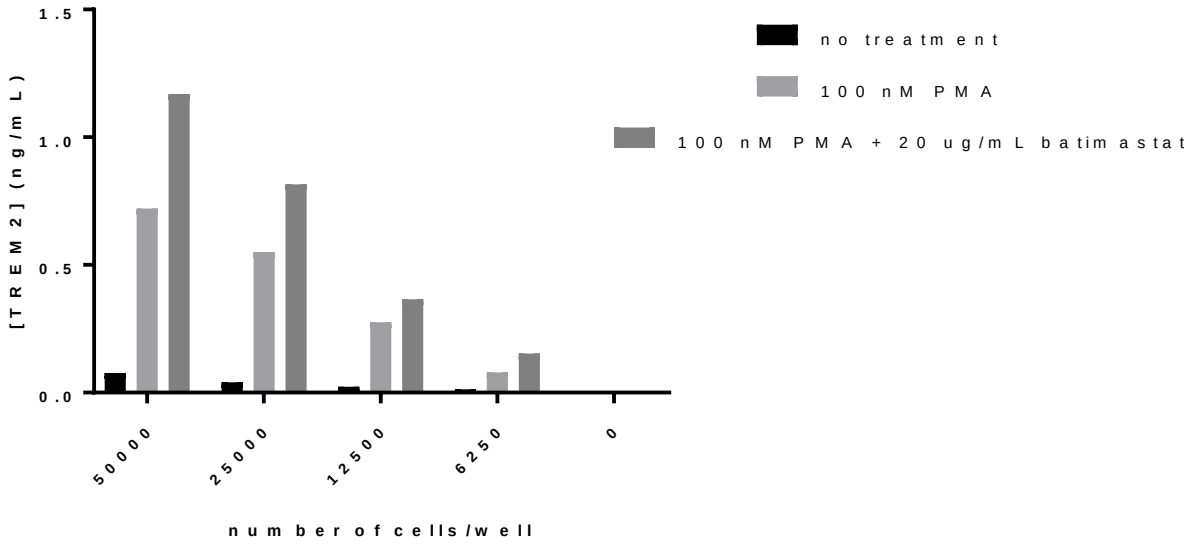


- Cell lysate assay

Cell growth and treatment:

1. A tube of frozen THP-1 cells was unfrozen and added to a T-75 flask with RPMI 1640 + 10% FBS.
2. Cells were grown until confluence (about 4 days).
3. Cells were harvested by centrifugation 5 minutes at 1200g.
4. Cells were washed twice with 20 mL cold sterile PBS.
5. Cells were resuspended in RPMI + 10% FBS and counted by the Trypan blue method.
6. Cells were plated in duplicate in the wells of a 96 well culture-treated plate.
7. Cells were plated first at 100 000 cells per well (1 000 000 cells per mL) in 100 uL
8. Cells were diluted in 1:2 sequential dilutions (10 dilutions) and a 0 cells control.
9. One series of cells was incubated alone.
10. One series of cells was incubated with 100 nM PMA
11. One series of cells was incubated with 100 nM PMA and 20 ug/mL batimastat.
12. The cells were incubated for 3 days at 37ºC with an atmosphere of 5% CO_2.
13. Supernatant was removed from each well and discarded.
14. Cells were washed twice with sterile PBS.
15. 100 uL of 1X AlphaLISA Lysis Buffer was added to each well.

Cell lysate of each well was tested in triplicate using the assay described in this document, with 1X AlphaLISA lysis buffer used as the diluent for the standard curve.



Troubleshooting Guide

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at: www.revvity.com

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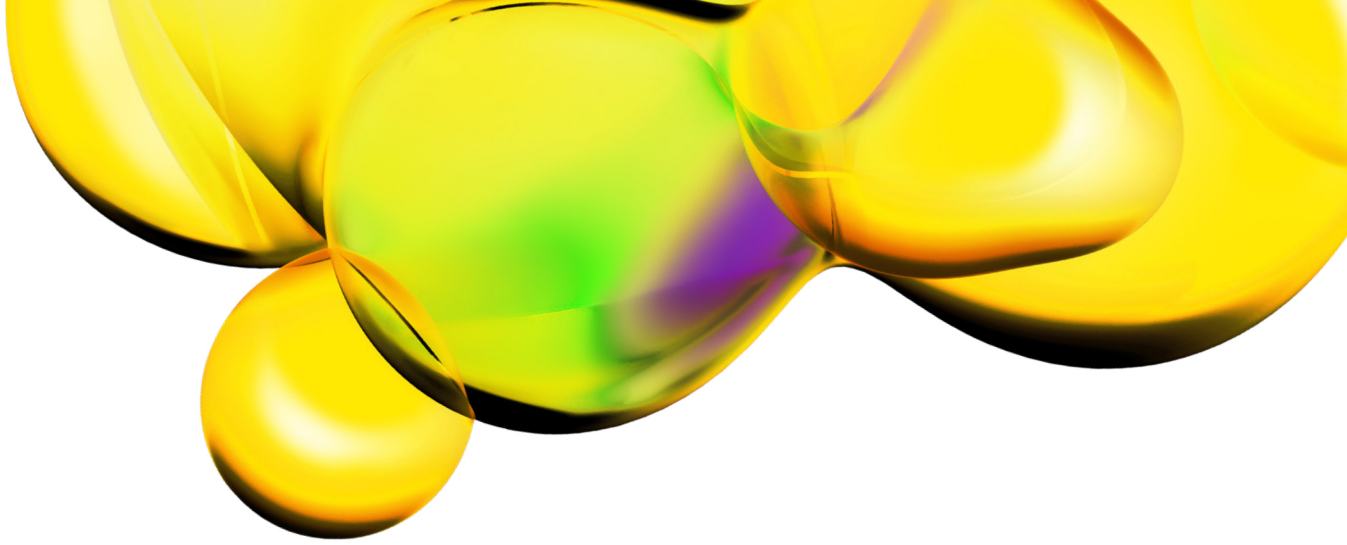

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