TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Research Use Only. Not for use in diagnostic procedures.

Human TREM2 AlphaLISA Detection Kit

Product No.: AL3109C/F

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Product Information

Application: This kit is designed for the quantitative determination of TREM2 in buffer, cell culture media, serum, plasma, cerebrospinal fluid and cell supernatant using a homogeneous AlphaLISA assay (no wash steps).

Sensitivity: Lower Detection Limit (LDL): 3.6 pg/mL

Lower Limit of Quantification (LLOQ): 12.5 pg/mL

EC_{50}: 15.7 ng/mL

Dynamic range: 3.6 – 100 000 pg/mL

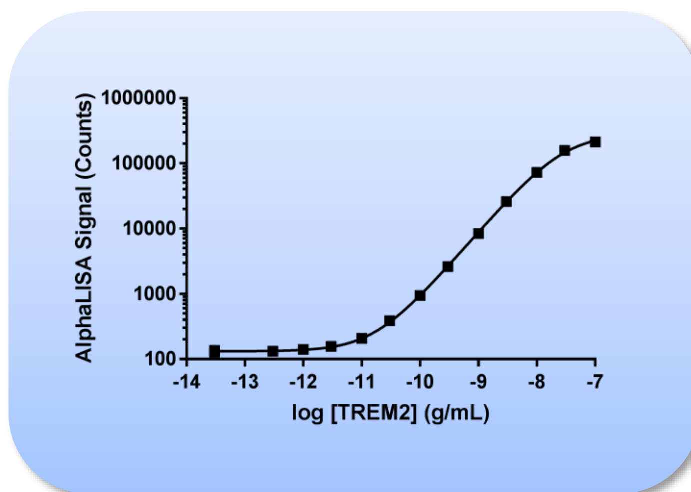

Figure 1. Typical sensitivity curve in AlphaLISA Universal Buffer. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader 2102 with Alpha option.

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

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

Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals, EC_{50} and LDL were measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in this technical data sheet. We certify that these results meet our quality release criteria. Maximum counts may vary between bead lots and the instrument used, with no impact on LDL measurement.

Analyte of Interest

TREM2 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, TREM2 has been shown to be involved in neurodegenerative diseases such as ataxia, early dementia and Alzeimer’s disease. Elevated levels of TREM2 have been noted in cerebrospinal fluid as a response to Alzeimer’s disease.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in buffer, cell culture media, human matrixes such as serum, plasma and cerebrospinal fluid, and cell supernatants in a highly sensitive, quantitative, reproducible and user-friendly mode. In this AlphaLISA assay, a biotinylated Anti-TREM2 Antibody binds to the Streptavidin-coated Alpha Donor beads, while another Anti-TREM2 Antibody is conjugated to AlphaLISA Acceptor beads. In the presence of the TREM2, the beads come into close proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 2).

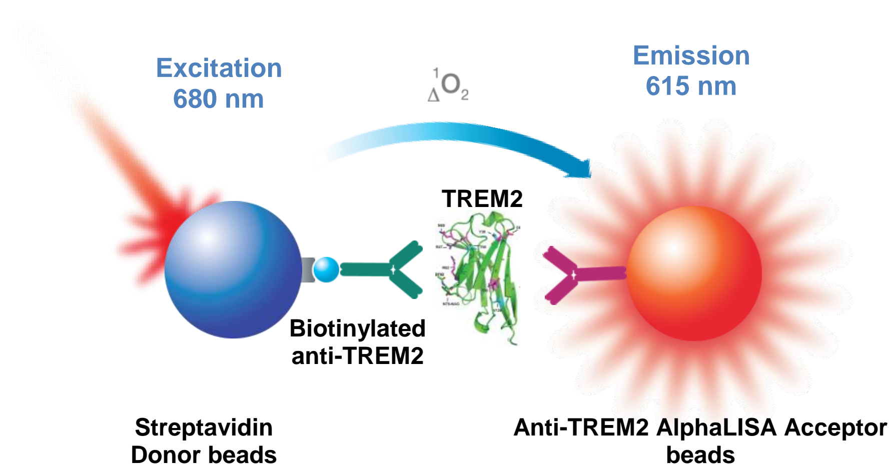

Figure 2. AlphaLISA TREM2 Assay Principle.

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-Analyte Antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Kit Content: Reagents and Materials

Kit components	AL3109HV (100 assay points***)	AL3109C (500 assay points***)	AL3109F (5000 assay points***)
AlphaLISA Anti-TREM2 Acceptor beads stored in PBS, 0.05% Proclin-300, 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% Proclin-300, 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-TREM2 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)
Recombinant TREM2, 10 ug/mL*	100 uL (1 tube, clear cap)	100 uL (1 tube, clear cap)	100 uL (1 tube, clear cap)
AlphaLISA Universal Buffer (5X)**	5 mL, 1 small bottle	20 mL, 1 medium bottle	2 x100 mL, 2 large bottles
AlphaLISA Lysis Buffer (5X)****	5 mL, 1 small bottle	5 mL, 1 small bottle	5 mL, 1 small bottle

* One vial contains an amount of analyte sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL3109S).

** Extra buffer can be ordered separately (cat # AL001C: 10 mL, cat # AL001F: 100 mL).

*** The number of assay points is based on an assay volume of 100 µL in HV size kits or 50 µL in C/F size kits using the kit components at the recommended concentrations.

**** Extra buffer can be ordered separately (cat # AL003C: 10 mL, cat # AL003F: 100 mL). This buffer is recommended for preparation of cell lysate samples.

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 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	PerkinElmer Inc.	6050185
EnVision®-Alpha Reader	PerkinElmer Inc.	-

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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 (2000g, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O to dilute 10X AlphaLISA Universal 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: D6 as, 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 protocol described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The protocols 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 protocol 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.

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Format	# of data points	Volume					Plate recommendation
		Final	Sample	AlphaLISA Acceptor Beads	Biotinylated Antibody	SA-Donor beads	
AL3109HV	100	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL3109C	250	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	50 µL	5 µL	10 µL	10 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) 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)
AL3109F	5 000	50 µL	5 µL	10 µL	10 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) 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)

3 Step Protocol 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 Universal Buffer:
   Add 10 mL of 5X AlphaLISA Universal Buffer to 40 mL Milli-Q® grade H_2O.

2) Preparation of TREM2 analyte standard dilutions:

   a. Dilute TREM2 analyte to 1 ug/mL by adding 10 uL to 90 µL of 1X Universal Buffer ***.
   b. Prepare standard dilutions as follows in 1X AlphaLISA Universal Buffer (change tip between each standard dilution):

Tube	Vol. of TREM2 (µL)	Vol. of diluent (µL)*	[TREM2] in standard curve

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			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of reconstituted TREM2	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 Universal Buffer, cell culture media or serum). The diluent used to dilute standards should match the sample type as closely as possible. We recommend preparing cell lysate samples and analyte dilutions used for analysis of cell lysate samples in 1X AlphaLISA lysis buffer (supplied in the kit as a 5X solution).
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).

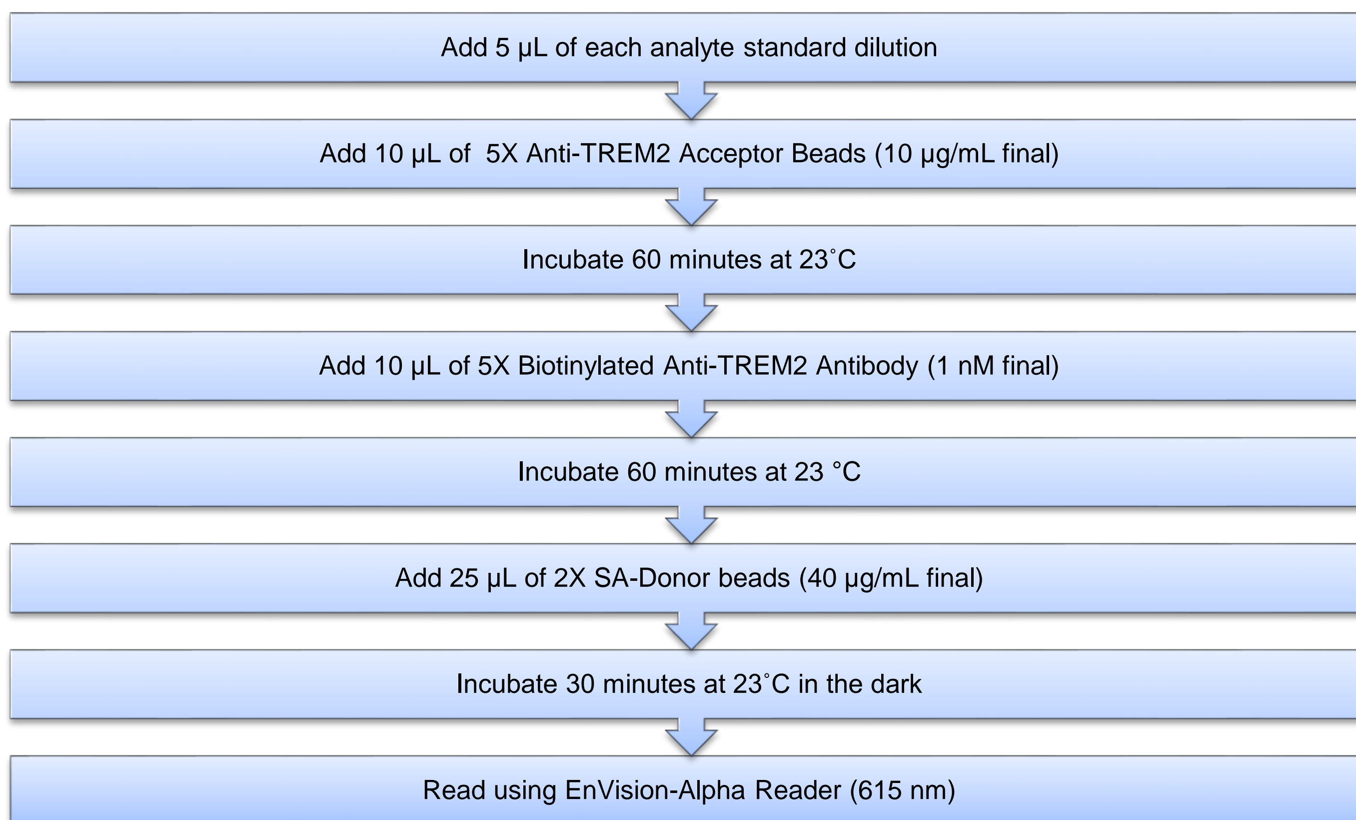
*** Excess of the 1 µg/mL dilution should be discarded after use.

3) Preparation of 5X Anti-TREM2 AlphaLISA Acceptor beads (50 µg/mL):
    a. Prepare just before use.
    b. Add 50 µL Anti-TREM2 Acceptor beads to 4950 µl of 1X AlphaLISA Universal Buffer.

4) Preparation of 5X biotinylated Anti-TREM2 antibody (5 nM):
    a. Prepare just before use.
    b. Add 50 µL 500 nM Biotinylated Anti-TREM2 Antibody to 4950 µl of 1X AlphaLISA Universal 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 200 µL of 5 mg/mL SA-Donor beads to 12 300 µL of 1X AlphaLISA Universal 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 using the 3 step protocol using AlphaLISA Universal Buffer as assay buffer. The analytes (standards) were prepared in Universal Buffer, DMEM, RPMI 1640, 100% FBS, 10% FBS in Universal Buffer and RPMI 1640 + 10% FBS and all other components were prepared in Universal Buffer.

- Assay Sensitivity:

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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
3.6	Universal Buffer	6
6.5	DMEM	6
29.0*	RPMI 1640	6
3.5	100% FBS	6
3.4	10% FBS in Universal Buffer	6
16.7	RPMI 1640 + 10% FBS	6

(*) Diluting RPMI 1640 1:1 with Universal buffer reduces LDL to 6.3 pg/mL

- 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 Universal Buffer, DMEM, RPMI 1640, 100% FBS or 10% FBS in Universal Buffer and RPMI 1640 + 10% FBS. All other components were prepared in Universal Buffer. 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 format.

- Intra-assay precision:

The intra-assay precision was determined using a total of 16 independent determinations in triplicate. Shown as CV%.

TREM2	Universal Buffer	DMEM	RPMI 1640	100% FBS	10% FBS in Universal Buffer	RPMI 1640 + 10% FBS
CV (%)	3	2	5	4	4	8

- Inter-assay precision:

The inter-assay precision was determined using a total of 3 independent determinations with 9 measurements for 10 ng/mL sample. Shown as CV%.

TREM2	Universal Buffer	DMEM	RPMI 1640	100% FBS	10% FBS in Universal Buffer	RPMI 1640 + 10% FBS
CV (%)	7	7	11	12	5	10

- Spike Recovery:

Three known concentrations of analyte were spiked into Universal Buffer, DMEM, RPMI 1640, 100% FBS or 10% FBS in Universal Buffer. All samples, including non-spiked diluents were measured in the assay. Note that the analytes for the

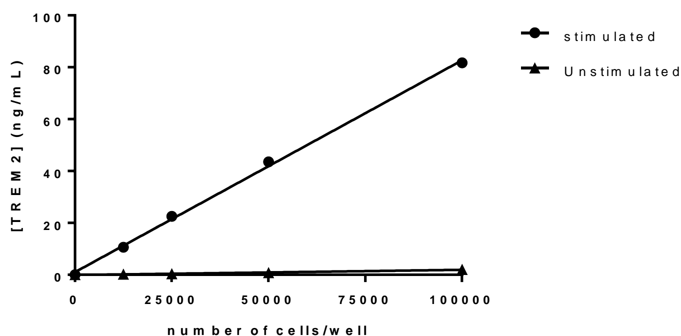
respective standard curves were prepared in Universal Buffer, DMEM, RPMI 1640, 100% FBS or 10% FBS in Universal Buffer and RPMI 1640 + 10% FBS. All other assay components were diluted in Universal Buffer.

Spiked TREM2 (ng/mL)	% Recovery					
	Universal Buffer	DMEM	RPMI 1640	100% FBS	10% FBS in Universal Buffer	RPMI 1640 + 10% FBS
10	91	110	88	110	93	112
3	97	89	87	114	94	86
1	102	92	101	107	87	97

- ## Cell Supernatant Experiments

THP-1 were grown in RPMI 1640 + 10% FBS. Cells were plated in a 96 well plate with amounts of 100 000, 50 000, 25 000 and 12 500 cells per well. One sample of cells was stimulated by incubation for 72 hours with phorbol myristate acetate (PMA) at 100 nM while the other sample was not treated. After incubation, cells were washed twice with sterile PBS and fresh culture media was added for 24 hours. The supernatant was collected and tested against a standard curve of TREM2 in RPMI 1640 + 10% FBS.

Cells per well	TREM2 detected in Stimulated Cell Supernatant (ng/mL)	TREM2 detection per cell (fg/mL)	TREM2 detected in Unstimulated Cell Supernatant (ng/mL)	TREM2 detection per cell (fg/mL)
100 000	81.7	817	2.0	20
50 000	43.5	870	0.8	16
25 000	22.5	900	0.38	16.9
12 500	10.6	848	0.18	14.4
0	0	0	0	0
Average	N/A	858	N/A	16.8



R^2 stimulated = 0.9985

R^2 unstimulated = 0.9885

- ## Serum and Plasma assays

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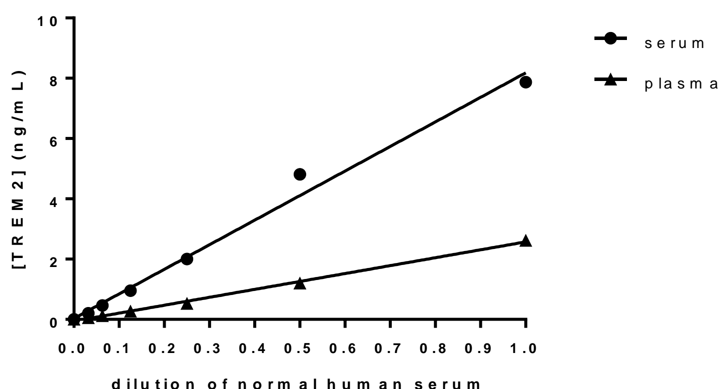
Samples of human serum or human plasma from healthy donors were used as is or diluted 1:2, 1:4, 1:8, 1:16 and 1:32 with Universal Buffer. Results were compared vs a standard curve made with Universal Buffer. Both human serum and plasma were filtered over 0.45 µm filters to remove lipid aggregates.

Dilution factor	Human serum (ng/mL)	Human serum corrected for dilution (ng/mL)	Human plasma (ng/mL)	Human plasma corrected for dilution (ng/mL)
raw	7.87	7.87	2.62	2.62
2	4.81	9.62	1.20	2.4
4	2.00	8.00	0.53	2.12
8	0.95	7.60	0.27	2.16
16	0.46	7.36	0.12	1.92
32	0.20	6.40	0.057	1.82
No matrix	0	0	0	0



R^2 serum = 0.9881

R^2 plasma = 0.9973

The kit can detect the protein with good linearity in raw matrix, and in matrix diluted up to 1:32 for both serum and plasma.

A spike and recovery experiment was performed on the matrixes. Both human serum and plasma were spiked with 10, 3 or 1 ng/mL of recombinant TREM2. A sample with no addition was used to measure basal levels and a sample of FBS was used as a control. All data were plotted against a standard curve performed in Universal Buffer.

Serum

Spike amount (ng/mL)	Observed amount (ng/mL)	Corrected amount (ng/mL)	% recovery
No spike	7.6	NA	NA
10	18	10.4	104
3	10.3	2.7	90
1	8.65	1.05	105

Plasma

Spike amount (ng/mL)	Observed amount (ng/mL)	Corrected amount (ng/mL)	% recovery

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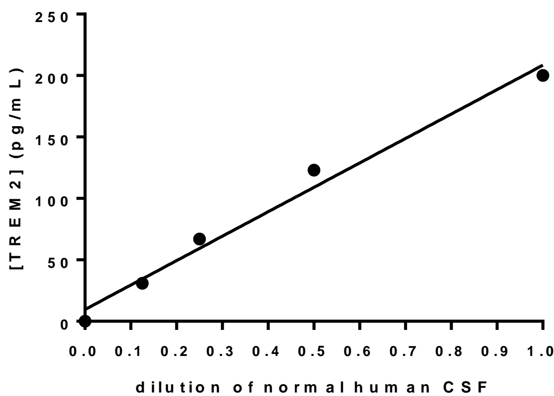
No spike	2.2	NA	NA
10	13.3	11.1	111
3	5.5	3.3	110
1	3.0	0.8	80

- **Results in Cerebrospinal fluid**

A sample of human cerebrospinal fluid from healthy donors was used as is or diluted 1:2, 1:4, and 1:8 with Universal Buffer. Results were compared vs a standard curve made with Universal Buffer.

Dilution factor	Observed (pg/mL)	Corrected for dilution
1	210	210
2	123	246
4	67	268
8	31	248



R^2 CSF = 0.9921

The kit can detect TREM2 in cerebrospinal fluid with good linearity.

A spike and recovery experiment was performed on cerebrospinal fluid. CSF was spiked with 10, 3 or 1 ng/mL of recombinant TREM2. A sample with no addition was used to measure basal levels. All data were plotted against a standard curve performed in Universal Buffer.

Spike amount (ng/mL)	Observed amount (ng/mL)	Corrected amount (ng/mL)	% recovery
No spike	0.21	NA	NA
10	11.3	11.1	111
3	3.8	3.6	120
1	1.0	0.8	80

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Troubleshooting Guide

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

http://www.perkinelmer.com/lab-products-and-services/application-support-knowledgebase/alphalisa-alphascreen-no-wash-assays/alpha-troubleshooting.html

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