MANUAL

AlphaLISA® Amyloid beta 1-40 HS Detection Kit

Product number: AL373C/F

Research Use Only. Not for use in diagnostic procedures.

Product Information

Application: This kit is designed for the quantitative determination of Amyloid beta 1-40 (Aβ40) in cerebrospinal fluid and cell culture supernatants using a homogeneous AlphaLISA assay (no wash steps). The assay shows negligible cross-reactivity with other forms of Amyloid beta (i.e. Aβ1-38 and Aβ1-42) .

Sensitivity: Lower Detection Limit (LDL): 42.2 pg/mL

Lower Limit of Quantification (LLOQ): 89.0 pg/mL

EC_{50}: 9.1 ng/mL

Dynamic range: Kit designed to detect [Aβ40] between: 42.2 – 10,000 pg/mL (Figure 1).

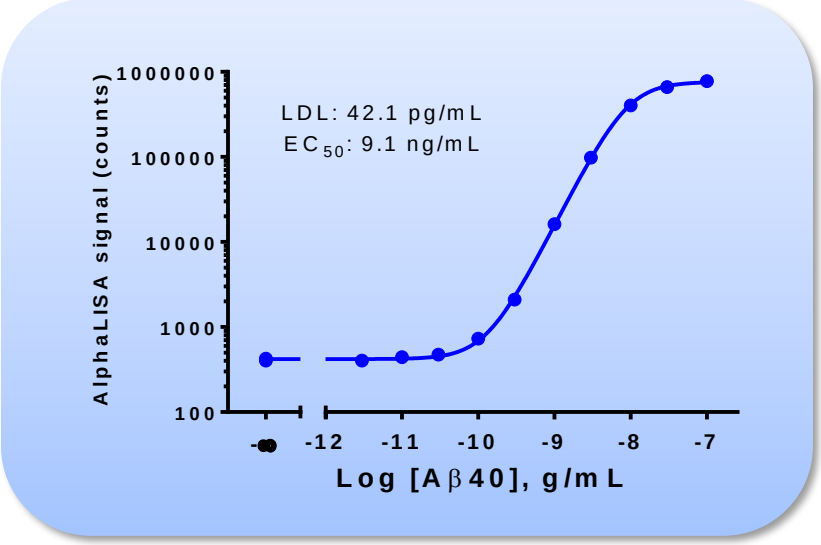


Figure 1. Typical sensitivity curve in AlphaLISA Immunoassay Buffer. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader 2103 with Alpha option.

Storage: Store kit in the dark at +4˚C. **Store analyte at -20°C.** Once the **Aβ 1-40 analyte** has been reconstituted, the analyte is stable for up to 6 weeks at 4˚C. For long term storage, aliquot and store at -20°C. Limit the number of freeze-thaw cycles.

Stability: This kit is stable for at least 12 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Analyte of Interest

Amyloid beta (Aβ) is a short peptide derived from a transmembrane protein, the amyloid precursor protein (APP), by proteolysis. The β- and γ-secretases cleave the respective N- and C-terminal ends of the Aβ sequence, liberating the Aβ peptide from APP. Aβ40 is the major species of Aβ produced by neurons and other cells, and accounts for over 70% of total Aβ produced, while the remainder is typically composed Aβ42 and other size variants ranging from 36 to 43 AA in length. Interestingly, it has been observed by some that the Aβ40/Aβ42 ratio increased in cerebrospinal fluid of AD patients. Aβ40 could be an important biomarker for detecting Alzheimer's disease progression. This AlphaLISA kit has been designed to detect Aβ40 in cell culture supernatants and cerebrospinal fluid.

Description of the AlphaLISA Assay

1AlphaLISA technology allows for the detection of molecules of interest in buffer, cell culture media, serum and plasma in a highly sensitive, quantitative, reproducible and user-friendly mode. In an AlphaLISA assay, a Biotinylated Anti-Analyte Antibody binds to the Streptavidin-coated Alpha Donor beads, while another Anti-Analyte Antibody is conjugated to AlphaLISA Acceptor beads. In the presence of the analyte, 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 transfers in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 2). Combining this assay with an AlphaPLEX 645- orAlphaPLEX 545 - based kit will allow the quantification of 2 (or more) analytes in the same well.

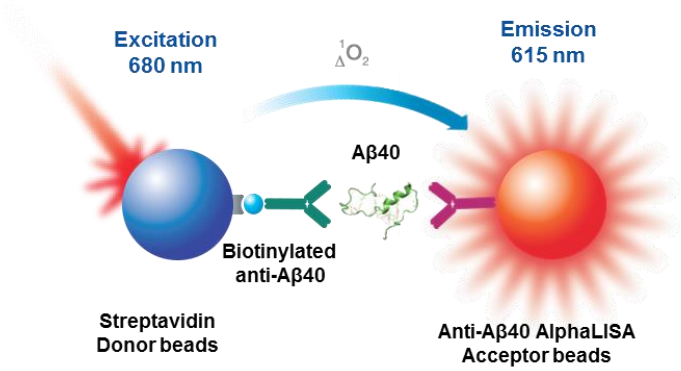


Figure 2. AlphaLISA assay principle.

Precautions

- The AlphaScreen® 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.
- All blood components and biological materials should be handled as potentially hazardous. The analyte included in this kit is from a source.
- Some analytes are present in saliva. 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	AL373C (500 assay points***)	AL373F (5000 assay points***)
AlphaLISA Anti-Aβ40 Acceptor beads stored in PBS, 0.05% Kathon, pH 7.2	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, pH 7.4	200 µL @ 5 mg/mL (1 brown tube, black cap)	2 x 1 mL @ 5 mg/mL (2 brown tubes, black caps)
Biotinylated Antibody Anti-Aβ40 stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	50 µL @ 500 nM (1 tube, black cap)	500 µL µL @ 500 nM (1 tube, black cap)
Amyloid-β 1-40 peptide*	20 µg, HFIP Film * (1 tube, clear cap)	20 µg, HFIP Film * (1 tube, clear cap)
AlphaLISA Immunoassay Buffer (10X) **	10 mL, 1 small bottle	100 mL, 1 large bottle

* Please note that the Amyloid-β 1-40 analyte film may not be visible to the naked eye. One vial contains an amount of Aβ40 sufficient for performing 100 standard curves. Additional vials can be ordered separately (cat # AL373S).

** 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 50 µL in 96- or 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 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 Adhesive Sealing Film	Revvity Inc.	6050195
EnVision®-Alpha Reader	Revvity Inc.	-
Dimethyl Sulfoxide	Sigma	D2650

Recommendations

- 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 (18 MΩ•cm) to dilute 10X AlphaLISA Immunoassay Buffer.
- 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.
- AlphaLISA signal is detected using an EnVision Multilabel Reader 2103 equipped with the Alpha option using the following settings: Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as (Barcode# 444), Emission Filter: Wavelength 570nm, bandwidth: 100nm, Transmittance 75%, (Barcode# 244).
- 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. The standard curve should be performed in AlphaLISA Immunoassay buffer.

Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The manual described below is an ***example*** for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations) and 452 samples. The manuals also include testing samples in 384 well plates. If different amounts of samples are tested, the volumes of all reagents must be adjusted accordingly, as shown in the table below. ****These calculations do not include excess reagents 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 beads / Biotin Antibody MIX**	**SA-Donor beads**	**Plate recommendation**
AL373C	250	100 µL	10 µL	10 µL	80 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	50 µL	5 µL	5 µL	40 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	2 µL	2 µL	16 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	10 µL	1 µL	1 µL	8 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL373F	5 000	50 µL	5 µL	5 µL	40 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	2 µL	2 µL	16 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	1 µL	1 µL	8 µL	Light gray AlphaPlate-1536 (cat # 6004350)

Manual for Aβ40 Total AlphaLISA Assay

2 Step Manual – Dilution of standards in 1X AlphaLISA Immunoassay Buffer or cell culture medium. The manual described below is for one standard curve (48 wells) and 452 sample wells. *If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.*

Steps for Preparing Reagents

1) Preparation of 1X AlphaLISA Immunoassay Buffer:
Add 5 mL of 10X AlphaLISA Immunoassay Buffer to 45 mL H_2O.

2) Preparation of Aβ40 analyte standard dilutions (**Do NOT vortex analyte!**):

   1. Reconstitute 20 µg **Aβ40-HFIP film** gently with 10 µL of Dimethyl sulfoxide (DMSO; Sigma-Aldrich D2650) by swirling the tip at the bottom of the tube and repeatedly pipetting up and down for 10 – 15 seconds. **Do not vortex the analyte!** Dilute dissolved Aβ40 film to 20 mg/µL with 990 µL of 1X AlphaLISA Immunoassay Buffer
      a. Store on ice immediately after reconstitution. The reconstituted **Aβ40** analyte film is stable up to 6 weeks at 4°C
      b. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer or cell culture medium (change tip between each standard dilution):

Tube	Vol. of Aβ40 (µL)	Vol. of diluent (µL) *	[Aβ40] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of provided Aβ40	190	1.00E-06	1 000 000
B	60 µL of tube A	140	3.00E-07	300 000
C	60 µL of tube B	120	1.00E-07	100 000
D	60 µL of tube C	140	3.00E-08	30 000
E	60 µL of tube D	120	1.00E-08	10 000
F	60 µL of tube E	140	3.00E-09	3 000
G	60 µL of tube F	120	1.00E-09	1 000
H	60 µL of tube G	140	3.00E-10	300
I	60 µL of tube H	120	1.00E-10	100
J	60 µL of tube I	140	3.00E-11	30
K	60 µL of tube J	120	1.00E-11	10
L	60 µL of tube K	140	3.00E-12	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

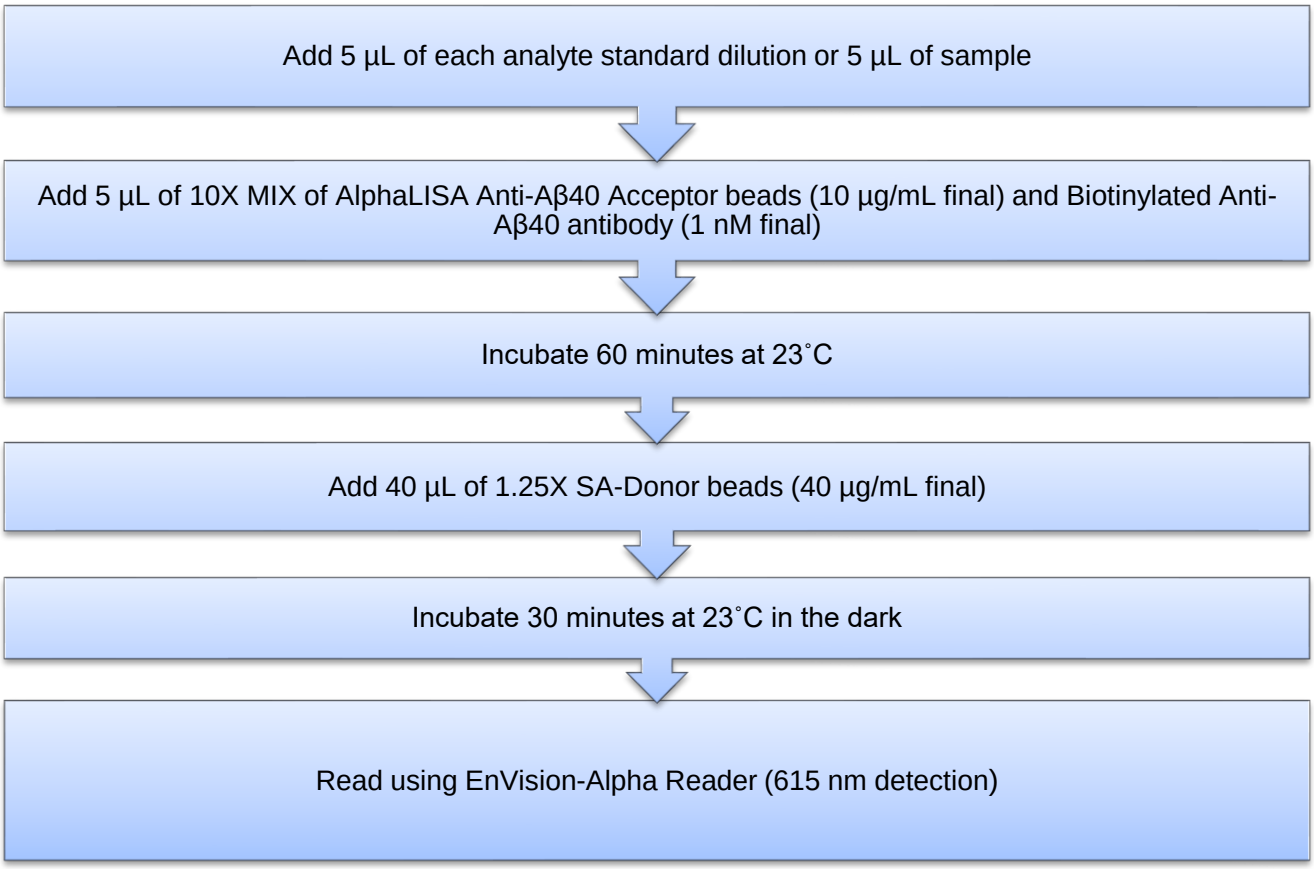
* Dilute standards in diluent (e.g. 1X AlphaLISA Immunoassay Buffer).
At low concentrations of analyte, a significant amount of analyte can bind to the vial. Therefore, load the analyte standard dilutions in the assay microplate within 60 minutes of preparation.

** 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 10X Anti-Aβ40 AlphaLISA Acceptor beads (100 µg/mL) and biotinylated Anti Aβ40 Antibody (10 nM):
    a. Add 50 µL of 5 mg/mL **AlphaLISA Anti-Aβ40 Acceptor beads and** 50 µL of 500nM **Biotinylated Anti-Aβ40 Antibody** to 2400 µL of 1X AlphaLISA Immunoassay Buffer .
    b. Prepare just before use.

4) Preparation of 1.25X Streptavidin (SA) Donor beads (50 µg/mL):
    a. Keep the beads under subdued laboratory lighting.
    b. Add 200 µL of 5 mg/mL SA-Donor beads to 19800 µL of 1X AlphaLISA Immunoassay Buffer.
    c. Prepare just before use.

5) In a white Optiplate (384 wells):



Read Settings: AlphaLISA signal is detected using an EnVision Multilabel Reader 2103 equipped with the Alpha option using the following settings: Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as (Barcode# 444), Emission Filter: Wavelength 570nm, bandwidth: 100nm, Transmittance 75%, (Barcode# 244).

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 2 step manual.

Assay Sensitivity:

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

LDL (pg/mL)	LLOQ (pg/mL)	Buffer/Serum	# of experiments
42	89	AlphaLISA Immunoassay Buffer	6
77	134	Neurobasal A	6
99	201	DMEM/F12	6

* Note that LDL/ LLOQ can be decreased (i.e. sensitivity increased) by increasing the volume of analyte in the assay (e.g. use 10 µL of analyte in a final assay volume of 50 µL).

** Only the analytes were prepared in Cell Culture media. All of other components were prepared in Immunoassay Buffer.

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 AlphaLISA Immunoassay Buffer (IAB), Neurobasal A medium, or DMEM / F12 medium. Each assay consisted of one standard curve comprising 12 data points in triplicate and 12 background wells containing no analyte. The assays were performed in a 384-well format using AlphaLISA Immunoassay Buffer.

- Intra-assay precision:

The intra-assay precision was determined using 3 independent experiments for a total of 16 independent determinations in triplicate. CV% were calculated for each individual experiment then averaged. Shown is the average intra-experimental CV%.

Aβ40	IAB	Neurobasal	DMEM/F12
CV%	5	8	8

- Inter-assay precision:

The inter-assay precision was determined using the data across 3 independent experiments with 16 measurements in triplicate. CV% was calculated by comparing the same measurement in each experiment. The CV% for all 16 measurements were then averaged. Shown is the inter-experimental CV%.

Aβ40	IAB	Neurobasal	DMEM/F12
CV%	13	23	21

- Spike Recovery:

Three known concentrations of Aβ40 were spiked into AlphaLISA Immunoassay Buffer (IAB), Neurobasal A medium, or DMEM/F12 medium. All samples, including non-spiked Immunoassay Buffers were measured in the assay. The average recovery was reported from 4 independent experiments each with 3 measurements in triplicate.

Spiked Aβ40 (ng/mL)	% Recovery		
	IAB	Neurobasal	DMEM/F12
3	95	80	87
1	87	76	86
0.3	88	80	84

- Specificity for Aβ40:

Cross-reactivity of the AlphaLISA Aβ40 Kit was tested against the following proteins and was calculated at the Aβ40 standard curve EC_{50} point when performed in AlphaLISA Immunoassay Buffer. Both monomers and aggregates of Aβ40 are detected by this assay kit.

Protein	% Cross-reactivity
Amyloid beta 1-38	0
Amyloid beta 1-42	0
Mouse/Rat Amyloid beta 1-40	0.2

Cerebrospinal Fluid Experiments

Pooled normal Cerebrospinal fluid (CSF) was utilized and AlphaLISA Immunoassay Buffer (IAB) was used as the diluent**.** Aβ40 was detected in the normal Human CSF (data not shown). Aβ40 is expected to be present at detectable levels in CSF from normal healthy subjects.

- Dilutional Linearity:

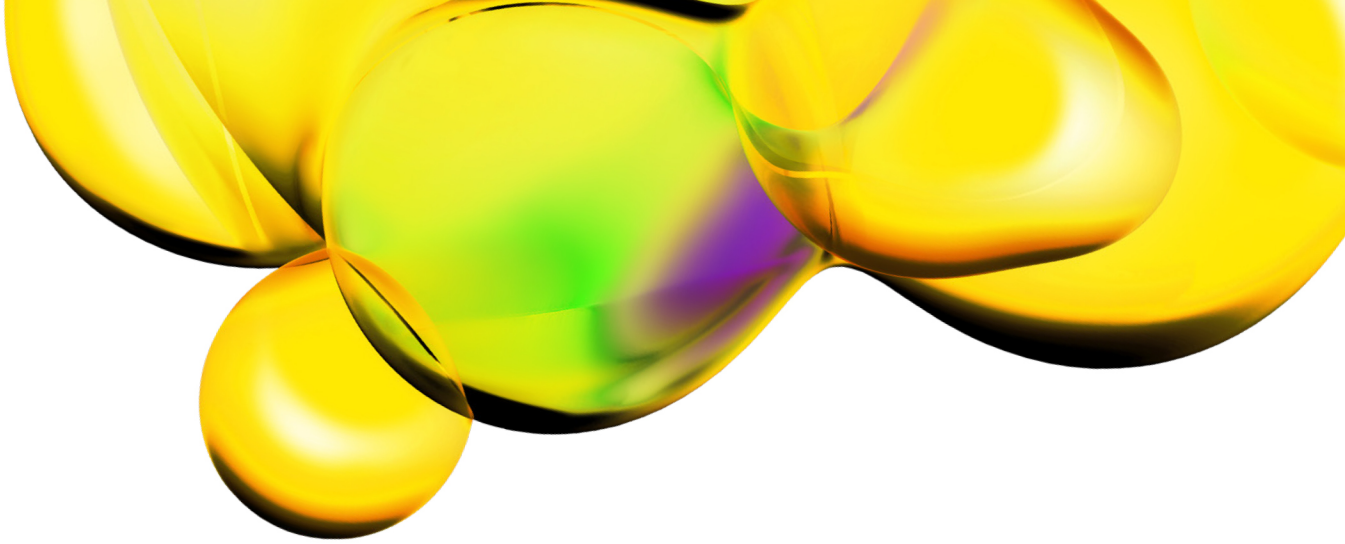
Dilutional linearity was determined by serial dilutions of Human CSF supplemented with 10 ng/mL of Aβ40 then diluted with IAB.

Dilution Factor	% Recovery
1	43%
2	60%
4	82%
8	95%
16	100%
32	102%
64	101%
128	90%
256	100%

Troubleshooting Guide

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

RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.



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