



AlphaLISA[®] S100A4 or FSP1 (Human) Detection Kit

Product number: AL3029 HV/C/F

Research Use Only. Not for use in diagnostic procedures.

Product Information

Application: This kit is designed for the quantitative determination of S100 calcium binding protein A4 (S100A4) in serum, plasma, CSF and cell lysates and cell culture supernatants using a homogeneous AlphaLISA assay (no wash steps). The assay shows negligible cross reactivity to S100B and S100A8/A9 (see page 10). The assay also detect mouse S100A4.

Sensitivity: Lower Detection Limit (LDL): 22.8 pg/mL
Lower Limit of Quantification (LLOQ): 82.7 pg/mL
EC₅₀: 140 ng/mL

Dynamic range: 22.8 – 300 000 pg/mL (Figure 1).

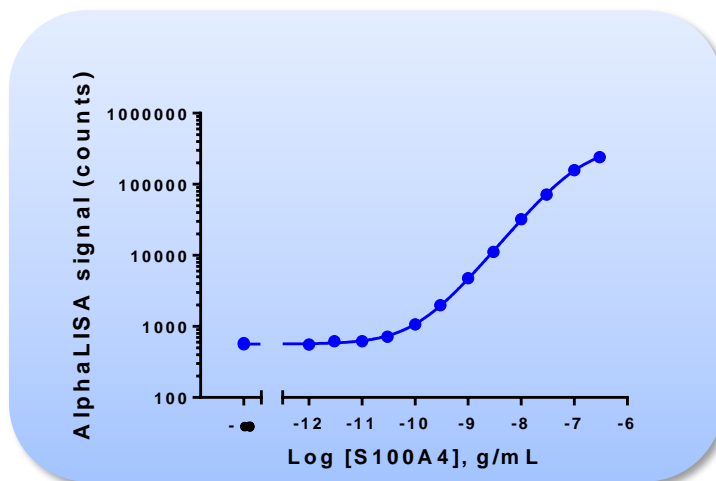


Figure 1. Typical sensitivity curve in AlphaLISA Immunoassay Buffer. The data was generated using a white Optiplate[™]-384 microplate and the EnVision[®] Multilabel Plate Reader 2103 with Alpha option.

Storage: Store kit in the dark at +4°C. Store reconstituted analyte at -20°C.

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.

Analyte of Interest

S100 calcium-binding protein A4 (S100A4) is a protein encoded by the S100A4 gene and is a member of the S100 family of proteins. In the cell, S100 proteins are localized mainly to the cytoplasm and the nucleus and regulate cellular processes. S100A4 has been well characterized for its role in cancer, particularly, enhanced cell growth and mobility leading to tumor progression and metastasis. This protein has been regarded a valuable biomarker since its expression level has been correlated with metastasis potential of many types of cancer, specifically, lung, prostate, breast and bone cancers. The S100A4 AlphaLISA kit was designed to detect S100A4 in serum, plasma, CSF, cell culture supernatants and cell lysates.

Description of the AlphaLISA Assay

AlphaLISA technology allows 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 transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 2).

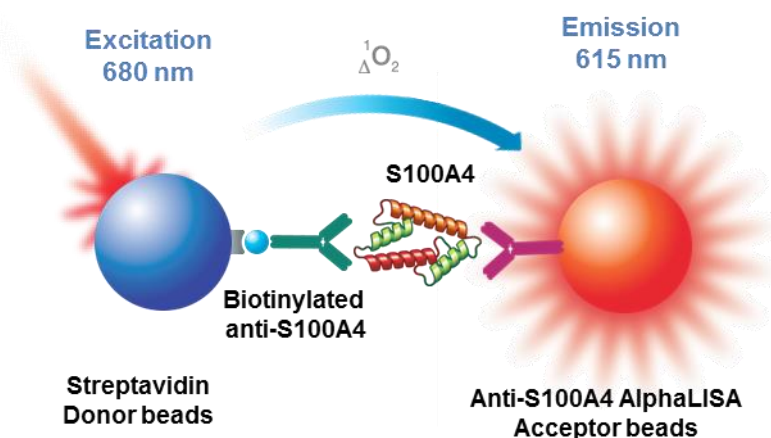


Figure 2. AlphaLISA Assay Principle.

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.
- All blood components and biological materials should be handled as potentially hazardous. The analyte included in this kit is from a human 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	AL3029HV (100 assay points ^{***})	AL3029C (500 assay points ^{***})	AL3029F (5000 assay points ^{***})
AlphaLISA Anti-S100A4 Acceptor beads stored in PBS, 0.05% Kathon, pH 7.2	20 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap)	50 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap)	500 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon, pH 7.4	80 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap)	200 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap)	2 x 1 mL @ 5 mg/mL (2 brown tubes, <u>black</u> caps)
Biotinylated Anti-S100A4 Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN ₃ , pH 7.4	20 µL @ 500 nM (1 tube, <u>black</u> cap)	50 µL @ 500 nM (1 tube, <u>black</u> cap)	500 µL @ 500 nM (1 tube, <u>black</u> cap)
Lyophilized S100A4 Analyte*	0.3 µg (1 tube, <u>clear</u> cap)	0.3 µg (1 tube, <u>clear</u> cap)	0.3 µg (1 tube, <u>clear</u> cap)
AlphaLISA Immunoassay Buffer (10X)**	2 mL, 1 small bottle	10 mL, 1 small bottle	100 mL, 1 large bottle

* Reconstitute S100A4 in 100 µL Milli-Q® grade H₂O. The reconstituted analyte should be used within 60 minutes or aliquoted into screw-capped polypropylene vials and stored at -20°C for further experiments. Avoid multiple freeze-thaw cycles. One vial contains an amount of S100A4 sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL3029S).

** 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 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 Plus Adhesive Sealing Film	Revvity Inc.	6050185
EnVision®-Alpha Reader	Revvity Inc.	-

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 H₂O (18 MΩ•cm) 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.
- The AlphaLISA signal is detected with an EnVision Multilabel 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. The standard curve should be performed in the AlphaLISA Immunoassay Buffer for serum and/or plasma samples.

Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- 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 a 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.

Format	# of data points	Final	Volume				Plate recommendation
			Sample	AlphaLISA Acceptor beads	Biotinylated Antibody	SA-Donor beads	
AL3029HV	100	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL3029C	250	100 µL	10 µL	s20 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)
AL3029F	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 a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.

- 1) Preparation of 1X AlphaLISA Immunoassay Buffer:
Add 10 mL of 10X AlphaLISA Immunoassay Buffer to 90 mL H₂O.

2) Preparation of S100A4 analyte standard dilutions:

- a. Reconstitute lyophilized S100A4 (0.3 µg) in 100 µL H₂O.
- b. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

Tube	Vol. of S100A4 (µL)	Vol. of diluent (µL) *	[S100A4] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of reconstituted S100A4	90	3.0E-07	300 000
B	60 µL of tube A	120	1.0E-07	100 000
C	60 µL of tube B	140	3.0E-08	30 000
D	60 µL of tube C	120	1.0E-08	10 000
E	60 µL of tube D	140	3.0E-09	3 000
F	60 µL of tube E	120	1.0E-09	1 000
G	60 µL of tube F	140	3.0E-10	300
H	60 µL of tube G	120	1.0E-10	100
I	60 µL of tube H	140	3.0E-11	30
J	60 µL of tube I	120	1.0E-11	10
K	60 µL of tube J	140	3.0E-12	3
L	60 µL of tube K	120	1.0E-12	1
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-S100A4 Antibody Acceptor beads (50 µg/mL):

- a. Prepare just before use.
- b. Add 50 µL of 5 mg/mL AlphaLISA Anti-S100A4 Antibody Acceptor to 4950 µL of 1X AlphaLISA Immunoassay Buffer.

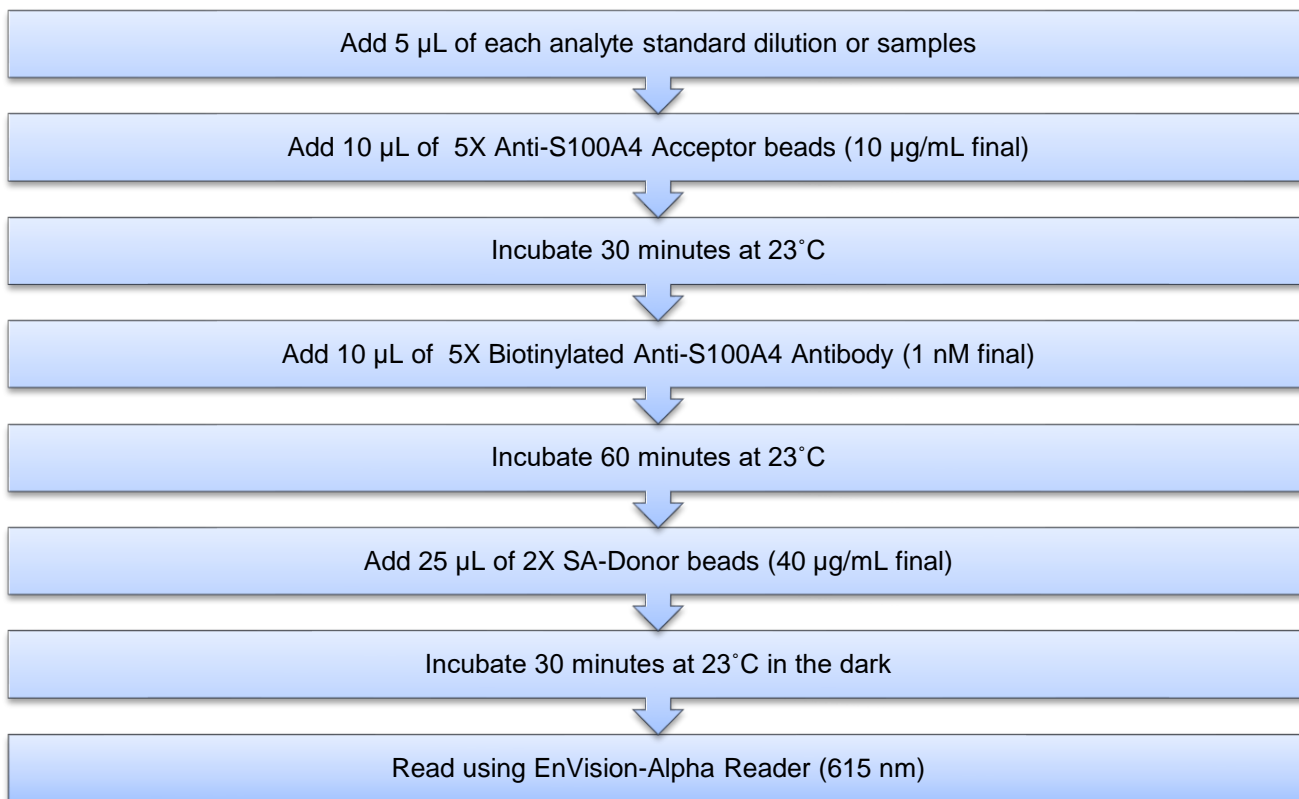
4) Preparation of 5X Biotinylated Anti-S100A4 Antibody (5 nM):

- a. Prepare just before use.
- b. Add 50 µL of 500 nM Biotinylated Anti-S100A4 Antibody to 4950 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 200 µL of 5 mg/mL SA-Donor beads to 12 300 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 using the 3 step protocol using AlphaLISA Immunoassay Buffer (IAB) and cell culture medium containing 10% FBS. In all cases assay components other than analyte were always prepared in IAB.

- 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 using the recommended assay conditions.

LDL (pg/mL)	Buffer/Serum/Medium	# of experiments
23	AlphaLISA Immunoassay Buffer	6
2.8	AlphaLISA Lysis Buffer	6
252	DMEM **	6
203	RPMI **	6

* The standard was prepared in these diluents. Note that LDL can be decreased (i.e. sensitivity increased) by preparing standards in different matrixes.

** Significant reduction in total counts was observed when compared to AlphaLISA Immunoassay Buffer. All samples in cell culture media must be compared to standard prepared in that media. Similar results were acquired when cell culture media was supplemented with 10% FBS, 1% BSA, or without additional supplements.

- 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 IAB, DMEM, or RPMI. 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 using IAB.

- Intra-assay precision:

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

S100A4	IAB	DMEM	RPMI	ALB
CV (%)	5	7	7	4

- Inter-assay precision:

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

S100A4	IAB	DMEM	RPMI	ALB
CV (%)	13	10	11	10

- Spike Recovery:

Three known concentrations of analyte were spiked in IAB, or in cell culture media. All samples, including non-spiked buffer or media were measured in the assay. The average recovery from three independent measurements is reported. Note that the standard curves were prepared in IAB, DMEM, and RPMI.

Spiked S100A4 (ng/mL)	% Recovery	
	IAB	ALB
10	97	102
3	103	102
1	102	101

Spiked S100A4 (ng/mL)	% Recovery	
	DMEM	RPMI
100	63	70
30	60	73
10	82	86

- Specificity:

Cross-reactivity of the S100A4 kit was determined by testing human S100A8/A9 and S100B and mouse S100A4. Cross reactivity was quantified at the Ec50 of the S100A4 standard curve.

Protein	% Cross-reactivity
Mouse S100A4	655
S100A8/A9	0.1
S100B	0.1

Human Serum, Plasma, and CSF Experiments

S100A4 was detected in serum, plasma, and CSF samples. Commercially available normal human samples were diluted in 2-fold increments with IAB. For all samples, at least 2- to 8-fold dilution with IAB is recommended.

Dilution Factor	Serum (recovery, ng/mL)	Plasma (recovery, ng/mL)	CSF (recovery, ng/mL)
2	8.5	7.8	0.37
4	7.1	6.8	0.48
8	7.6	6.6	0.55
16	8.7	6.7	0.67
32	9.5	7.2	0.69

Cell Lysate Experiments

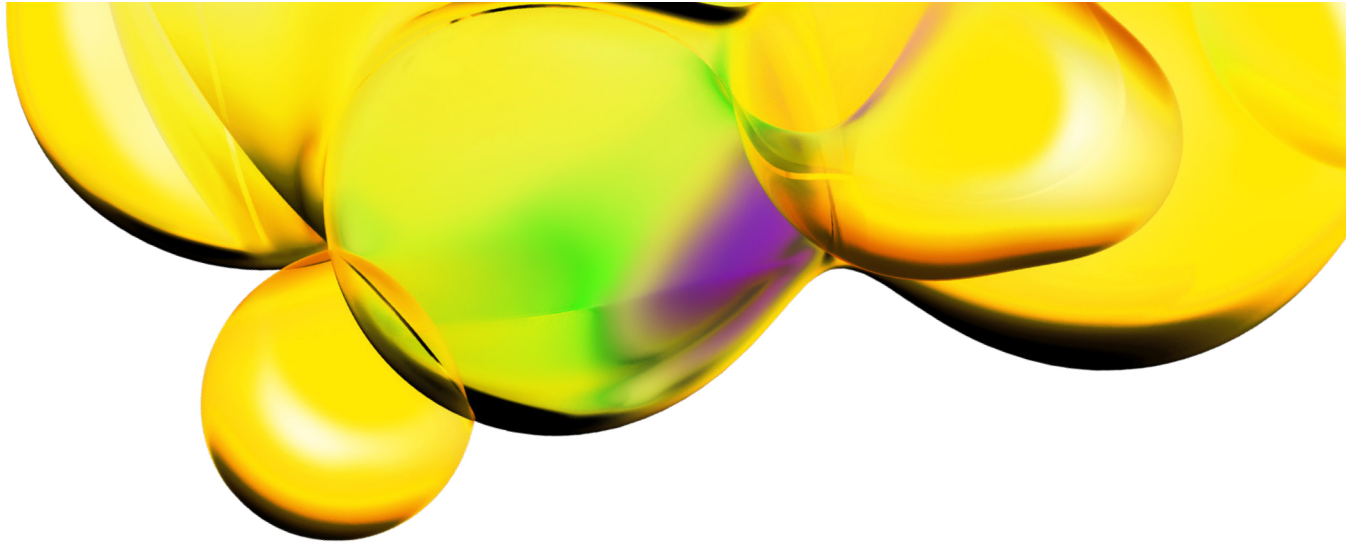
Commercially available control and S100A4 overexpressed HEK293 cell lysates were tested. The lysates were prepared using RIPA buffer. AlphaLISA lysis buffer (ALB) can also be used for cell lysates. Cell lysates were diluted in ½ log increments in AlphaLISA Lysis buffer and compared to an ALB standard curve (IAB can also be used as the diluent). Note: all beads and antibodies were diluted in IAB.

Total Protein (ng/mL)	S100A4 detected in Control Lysate (pg/mL)	% Total protein in Control Lysate	S100A4 detected in overexpression Lysate (pg/mL)	% Total protein in Overexpression Lysate
10 000	8.8	0.00008	Greater than UDL	-
3 000	2.1	0.00007	Greater than UDL	-
1 000	0.9	0.00008	Greater than UDL	-
300	Below LDL	-	7 697	2.6
100	Below LDL	-	3 086	3.1
30	Below LDL	-	944	3.2
10	Below LDL	-	311	3.1

Troubleshooting Guide

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

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