AlphaLISA Soybean Agglutinin Detection Kit

Product number: AL404 HV/C/F

Lot number: sample

Manufacturing date:

Caution: For Laboratory Use. A research product for research purposes only.

Contents

Product Information

Application: This kit is designed for the quantitative determination of Soybean agglutinin in food products in order to detect the presence of soybean proteins. It shows negligeable cross-reactivity with other lectins from alimentary plants.

Sensitivity: Lower Detection Limit (LDL): 9.5 pg/mL (or 9.5 ppb)

Lower Limit of Quantification (LLOQ): 35 pg/mL

EC_{50}: 180 ng/mL

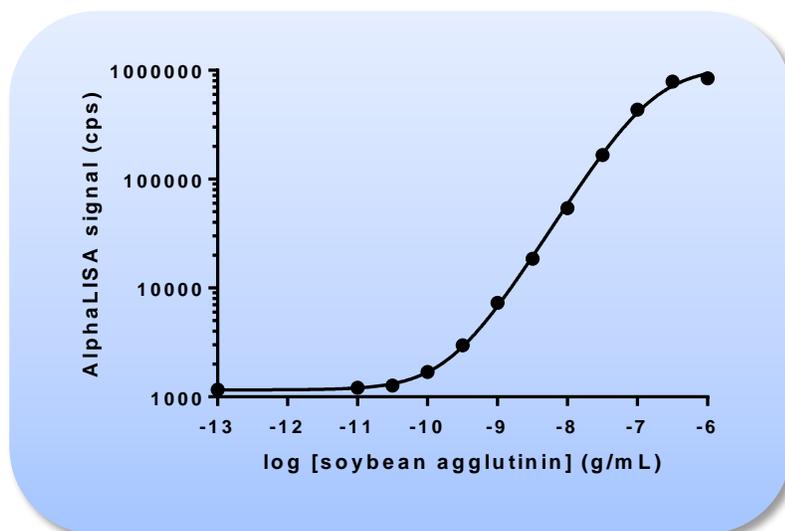**Dynamic range:**   10 – 1 000 000 ng/mL

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

Storage: Store kit in the dark at +4˚C. For analyte aliquot and store at -80 °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.

EC_{50}:	261.3 ng/mL
LDL:	15.54 pg/mL
LLOQ	84.68 pg/mL
Min Counts:	397
Max Counts:	182901

Analyte of Interest

Soybean and its extracts are considered a major part of the world food production. Soybean proteins are used in many products ranging from cultural cuisine such as Asian food (sprouts, soy sauce) and non-meat products (tofu, vegan products). Unfortunately, some of its proteins have been shown to cause allergic in many individuals. This creates a need to monitor the presence of soy products in food. Agglutinin is a particularly dangerous product since it can prove toxic by several mechanisms such as being a potent allergen, binding to intestinal cell surfaces and generating inflammation and even by chelating essential heavy metal in the intestinal lumen. This kit is designed to detect and quantify soybean agglutinin in food products.

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).
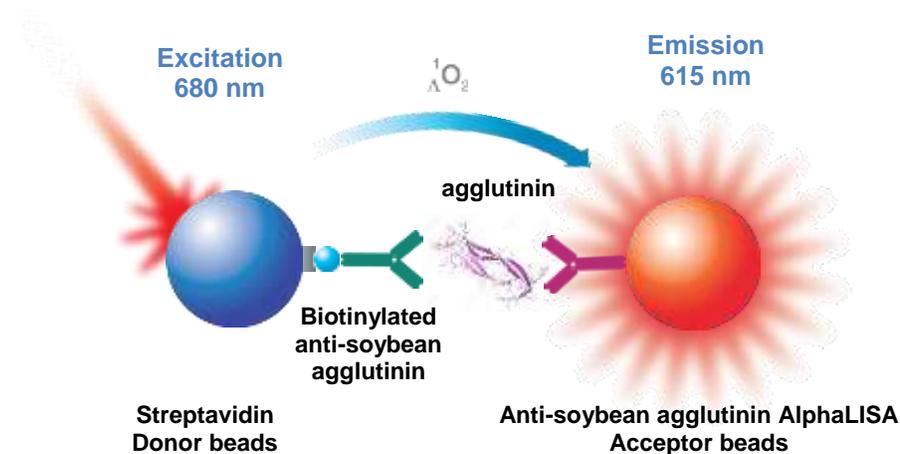
Precautions

- 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	AL404HV (100 assay points**)	AL404C (500 assay points**)	AL404F (5000 assay points**)
AlphaLISA Anti- soybean agglutinin 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-soybean agglutinin 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)
Soybean agglutinin (10 µg/mL) in PBS + 0.05% ProClin 300	100 µL (1 tube, clear cap)	100 µL (1 tube, clear cap)	100 µL (1 tube, clear cap)
AlphaLISA Immunoassay Buffer (10X)*	2 mL, 1 small bottle	10 mL, 1 medium bottle	100 mL, 1 large bottle

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

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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.

- 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.

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.

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Format	# of data points	Final	Sample	AlphaLISA Acceptor beads and Biotinylated Antibody	SA-Donor beads	Plate recommendation
				Volume		
AL404HV	100	100 µL	10 µL	40 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL404C	250	100 µL	10 µL	40 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	50 µL	5 µL	20 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	8 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	4 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL 404F	5 000	50 µL	5 µL	20 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	8 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	4 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

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2 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, <u>the volumes of all reagents have to be adjusted accordingly</u>.*

1) Preparation of 1X AlphaLISA Immunoassay Buffer:

      a. Add 5 mL of 10X AlphaLISA Immunoassay Buffer to 45 mL H_2O.

2) Sample preparation from food material

      a. Homogenize food material.
      b. Add 12 volumes of water (i.e. w/v)
      c. Adjust pH to 4.5 with 0.1 N HCl
      d. Incubate at $4^°C$ for 10 minutes with constant agitation.
      e. Centrifuge 1 ml of material for 5 minutes at 10,000 x g on a table top centrifuge.
      f. Keep supernatant for analysis. The pellet can be discarded.
      g. It is recommended to perform a set of dilutions of the sample for analysis.

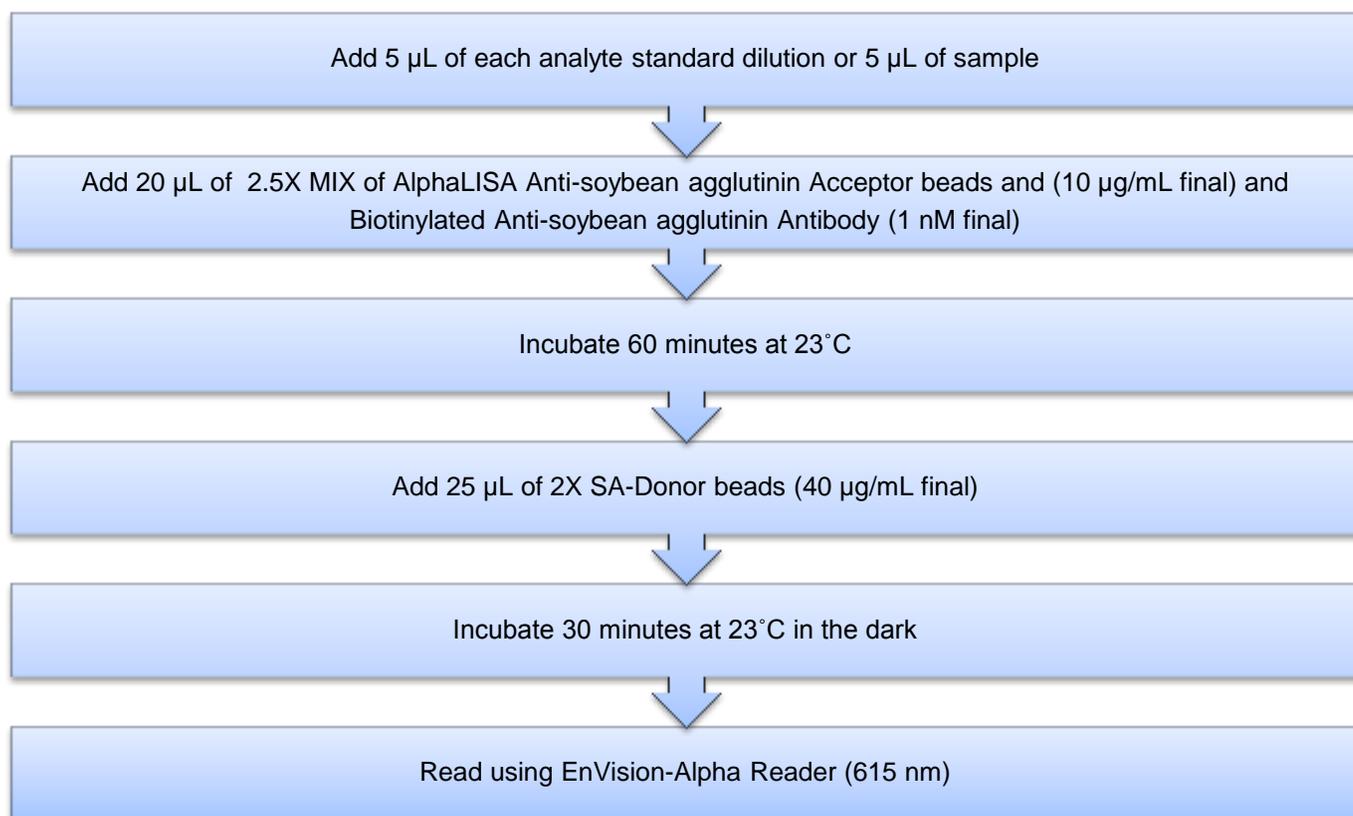3) Preparation soybean agglutinin analyte standard dilutions:
      a. Thaw Soybean agglutinin analyte (10µg/mL)
      b. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

Tube	Vol. of soybean agglutinin (µL)	Vol. of diluent (µL) *	[soybean agglutinin] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of soybean agglutinin	90	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).

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4) Preparation of 2.5X MIX AlphaLISA Anti-soybean agglutinin Acceptor beads (25 µg/mL) and Biotinylated Anti-soybean agglutinin antibody (2.5 nM):
   a. Prepare just before use.
   b. Add 50 µL of 5 mg/mL AlphaLISA Anti-soybean agglutinin Antibody Acceptor and 50 µL of Biotinylated Anti-soybean agglutinin antibody to 9900 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 12300 µL of 1X AlphaLISA Immunoassay Buffer.

6) In a white Optiplate (384 wells):

Add 5 µL of each analyte standard dilution or 5 µL of sample

Add 20 µL of 2.5X MIX of AlphaLISA Anti-soybean agglutinin Acceptor beads and (10 µg/mL final) and Biotinylated Anti-soybean agglutinin Antibody (1 nM final)

Incubate 60 minutes at 23˚C

Add 25 µL of 2X SA-Donor beads (40 µg/mL final)

Incubate 30 minutes at 23˚C in the dark

Read using EnVision-Alpha Reader (615 nm)

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).

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- 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 protocol using AlphaLISA Immunoassay Buffer (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 (ng/mL)	Buffer *	# of experiments
9.5	IAB	6

* The standard was prepared in these diluents and all other components were diluted in IAB. Note that LDL 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).

- 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. All other components were also prepared in IAB. 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 by averaging 6 experiments each with 12 independent determinations in triplicate. Shown as CV%.

soybean agglutinin	IAB
CV (%)	6.4

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- Inter-assay precision:

The inter-assay precision was determined comparing 6 experiments each with 12 independent determinations in triplicate. Shown as CV%.

soybean agglutinin	IAB
CV (%)	15.4

- Spike Recovery:

Known concentrations of analyte were spiked into IAB. All samples, including non-spiked buffer were measured in the assay. All components were diluted in IAB.

Spiked soybean agglutinin (ng/mL)	IAB
300	108
100	116
30	95

- Specificity:

Cross-reactivity of the Soybean Agglutinin AlphaLISA Kit was tested using the following proteins at 10 µg/mL in IAB.

Lectin	Common name	% Cross-reactivity
Triticum vulgaris	Wheat	0
Ricinus communis	Ricin	0
Dolichos biflorus	Dolichos seed	0
Arachis hypogaea	Peanut	0
Glycine max	Soybean	100
Ulex europaeus	Gorse	0
Psophocarpus tetragonolobus	Winged bean	0
Musa paradisica	Banana	<0.1%
Solanum tuberosum	Potato	0.1%
Sophora japonicum	Pagoda tree	0
Griffonia Simplicifolia	Griffonia tea	0.1%
Pisum sativum	Green pea	<0.1%
Lens cunilaris	Lens	<0.1%
Phaseolus vulgaris (erithroagglutinin)	Common bean	0
Phaseolus vulgaris (leucoagglutinin)	Common bean	0
Concanavalin A	Jack Bean	0

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Food Experiments

To validate the assay kit, commercially available soy flour and soy-free products were used:

Unknown concentrations of soybean agglutinin were detected in soy flour. Soybean agglutinin levels detected were similar to those reported in the literature (about 1.35% of total protein). It is recommended to dilute your samples at least 30 000-fold for proper recovery.

Troubleshooting Guide

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

http://www.perkinelmer.com/in/resources/technicalresources/applicationsupportknowledgebase/alphalisa-alphascreen-no-washassays/alpha_troubleshoot.xhtml

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