TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Research Use Only. Not for use in diagnostic procedures.

AlphaLISA SARS-CoV2 Spike S1 Protein Detection Kit

Product No.: AL3142

Contents

Product Information

Application: This kit is designed for the quantitative determination of SARS-Cov2 Spike S1 protein using a homogeneous no wash AlphaLISA assay.

Kit contents: The kit contains six components: AlphaLISA Acceptor beads coated with anti-Spike S1 Antibody, Streptavidin-coated Donor beads, Biotinylated anti-Spike S1, frozen Spike S1 standard, 10X AlphaLISA Immunoassay Buffer, and 5X AlphaLISA Lysis Buffer.

Sensitivity: Lower Detection Limit (LDL): 34 pg/mL
Lower Limit of Quantification (LLOQ): 104 pg/mL
EC_{50}: 24.7 ng/mL

Dynamic Range: 24.7 – 100 000 pg/mL

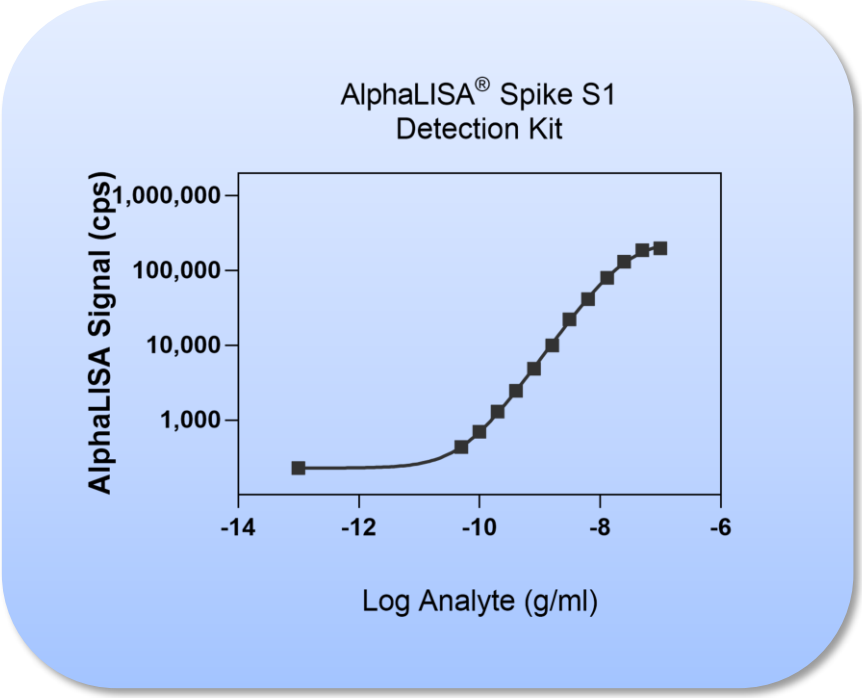

Figure 1. Typical sensitivity curve in AlphaLISA Immunoassay Buffer. The data was generated using a white Proxiplate™-384 microplate and the EnVision® Multilabel Plate Reader 2102 with Alpha option.

Storage: Store kit in the dark at 4 ºC, Store frozen analyte 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.

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Analyte of Interest

The SARS-CoV-2 Spike S1 protein is one of two part of the virus Spike protein (S). The S1 subunit is responsible for the the virus interaction with host cells' transmembrane Angiotensin-converting enzyme 2 receptors (ACE2), while the S2 subunit is capable of conformational changes that allow the fusion of the viral particle envelope and host cell membrane upon S1/ACE2 binding. As such, the S1 protein is closely investigated for its role in viral infection and is reliable marker of infection as well as a potential vaccine immunogen target.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in cell lysates in a highly sensitive, quantitative, reproducible and user-friendly mode. In this AlphaLISA assay, a biotinylated anti-Spike S1 antibody binds to the streptavidin coated AlphaLISA Donor beads, while the anti-Spike S1 antibody is conjugated to AlphaLISA Acceptor beads. In the presence of Spike S1 protein, 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).

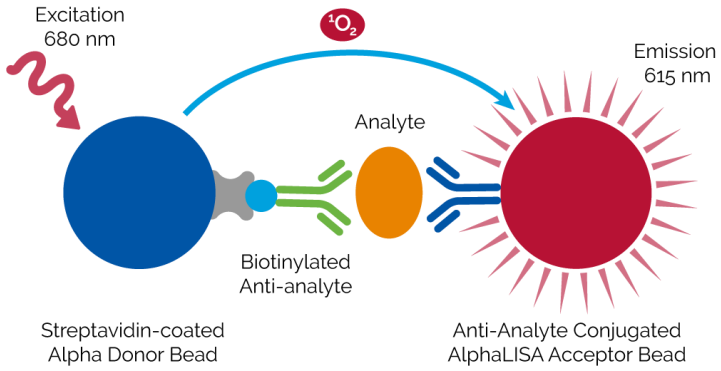


Figure 2. AlphaLISA SARS-CoV2 Spike S1 Protein Detection Assay Principle.

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-Spike S1 antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Kit Content: Reagents and Materials

Kit components	AL3142C 500 assay points***	AL3142F 5000 assay points***
AlphaLISA Anti-Spike S1 Acceptor beads stored in PBS, 0.05% Kathon CG/ICP, pH 7.2	40 µL @ 5 mg/mL (1 brown tube, white cap)	400 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	40 µL @ 5 mg/mL (1 brown tube, black cap)	400 µL @ 5 mg/mL (1 brown tube, black cap)
Biotinylated Anti-Spike S1 Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	10 µL @ 500 nM (1 tube, black cap)	100 µL @ 500 nM (1 tube, black cap)
Frozen Spike S1 Analyte* (0.25 mg/mL)	10 µL (1 tube, clear cap)	10 µL (1 tube, clear cap)
AlphaLISA Immunoassay Buffer (10X) **	2 mL, 1 small bottle	10 mL, 1 small bottle
AlphaLISA Lysis Buffer (5X)**	2 mL, 1 small bottle	10 mL, 1 small bottle

* Store Frozen Analyte at -20°C. 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 # AL3142S).

** Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL for 10X Immunoassay Buffer or cat# AL003C: 10 mL, cat# AL003F: 100 mL for 5X Lysis Buffer).

*** The number of assay points is based on an assay volume of 20 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-Spike S1 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.	-

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 protocol described below is an example for generating one standard curve in a 20 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.

		Volume				
Format	**# of data points**	**Final**	**Sample**	**MIX AlphaLISA AccBeads + biotinylated Ab**	**SA-Donor beads**	**Plate recommendation**
AL3142C	250	40 µL	10 µL	20 µL	10 µL	½ Area AlphaPlate-96 (cat # 6002350) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	500	20 µL	5 µL	10 µL	5 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	1 250	10 µL	2.5 µL	5 µL	2.5 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL3142F	2500	40 µL	10 µL	20 µL	10 µL	½ Area AlphaPlate-96 (cat # 6002350) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	5000	20 µL	5 µL	10 µL	5 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	10000	10 µL	2.5 µL	5 µL	2.5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

The 2-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 Immunoassay Buffer:
Add 5 mL of 10X AlphaLISA Immunoassay Buffer to 45 mL Milli-Q® grade H_2O.

2) Preparation of Spike S1analyte standard dilutions:

a. Prepare Dilution 1: Dilute stock solution (0.25 mg/mL) of Spike S1 protein 100-fold (e.g 5uL of stock in 495uL of 1X AlphaLISA Lysis Buffer). Dilution 1 yields a 2,500 ng/mL solution.
b. Prepare standard dilutions as follows in 1X AlphaLISA Lysis Buffer (change tip between each standard dilution):

Tube	Vol. of Spike S1 (µL)	Vol. of diluent (µL) *	[Spike S1] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	8 µL of Dilution 1	192	1E-07	100
B	100 µL of tube A	100	5E-08	50
C	100 µL of tube B	100	2.5E-08	25
D	100 µL of tube C	100	1.25E-08	13
E	100 µL of tube D	100	6.25E-09	6.3
F	100 µL of tube E	100	3.13E-09	3.1
G	100 µL of tube F	100	1.56E-09	1.6
H	100 µL of tube G	100	7.81E-10	0.8
I	100 µL of tube H	100	3.91E-10	0.4
J	100 µL of tube I	100	1.95E-10	0.2
K	100 µL of tube J	100	9.77E-11	0.10
L	100 µL of tube K	100	4.88E-11	0.05
M ** (background)	0	50	0	0
N ** (background)	0	50	0	0
O ** (background)	0	50	0	0
P ** (background)	0	50	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).

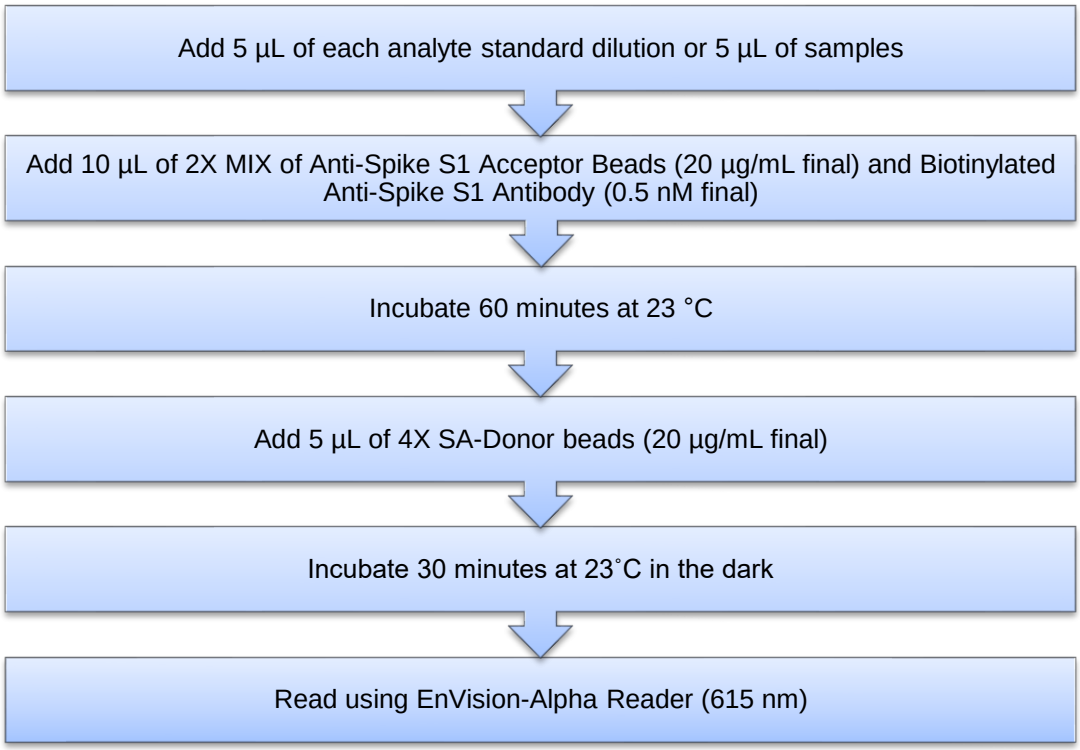
3) Preparation of 2X MIX of AlphaLISA Anti-Spike S1 Acceptor beads (40 µg/mL) and Biotinylated Anti-Spike S1 Antibody (1 nM):

a. Prepare just before use.
b. Add 16 µL of 5 mg/mL AlphaLISA Anti-Spike S1 Acceptor beads and 4 µL of 500 nM to Biotinylated Anti-Spike S1 Antibody to 1980 µL of 1X AlphaLISA Immunoassay Buffer. Mix briefly.

4) Preparation of 4X 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.

5) In a white Proxiplate (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.

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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