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## **Anti-Human IgG Fab AlphaLISA Acceptor Bead ToolBox**

Product number: AL177

Lot number: 2855251

Manufacturing date: January 26, 2021

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## Product Information

**Description:** Anti-Human IgG Fab AlphaLISA Acceptor Beads at 5 mg/mL in PBS pH 7.2 supplemented with 0.05% Kathon CG/ICP as a preservative. The antibody utilized is a mouse IgG2b Monoclonal.

**Application:** This product is intended for use in homogenous Alpha assays to capture human IgG Fab fragment.

**Specificity:** This product has minimum cross reactivity (<1%) with human IgG Fc, IgA, IgM and mouse, rat, rabbit, and goat IgG.

### Formats:

Catalog #	Size	Volume	Assay Points
AL177C	250 µg	50 µL	500
AL177M	5 mg	1000 µL	10 000
AL177R	25 mg	5000 µL	50 000

\* The number of assay points is based on an assay volume of 25 µL using a final bead concentration of 20 µg/mL in 384-well format

**Storage:** Store at 4 °C.

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

## Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum, minimum signals, and EC<sub>50</sub> were measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in this technical data sheet. We certify that the results meet our quality release criteria. Note: maximum counts will vary depending on assay conditions as well as between lots and instrument used. This variation has no impact on assay quality.

Maximum Counts: 448,089 counts  
Minimum Counts: 366 counts  
EC<sub>50</sub>: 127.20 ng/mL

## 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 before use to improve recovery of content (2000g, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H<sub>2</sub>O (18 MΩ•cm) to dilute 10X AlphaLISA Immunoassay Buffer.
- When diluting the probe, 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 Plus Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Plus 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.

## Quality Control Protocol

This protocol provides a means to verify product performance. It is used as our Quality Control release test. The following reagents and materials are used in addition to the Acceptor Beads:

Kit components	Suggested Source	Catalog #
AlphaScreen Streptavidin Donor beads	PerkinElmer	6760002S (1 mg) 6760002 (5 mg) 6760002B (50 mg)
Biotin-Human IgG, Fab fragment	Jacksonimmuno	009-060-007
White OptiPlate™-384	PerkinElmer	6007290
TopSeal™-A Plus Adhesive Sealing Film	PerkinElmer	6050185
AlphaLISA Immunoassay Buffer (10X)	PerkinElmer	AL000C (10 mL) AL000F (100 mL)
EnSpire® or EnVision® Multilabel Alpha Reader	PerkinElmer	-

## Assay Protocol

This protocol is designed for 12 dilutions of the probe with triplicate determinations. Final concentration of anti- human IgG Fab AlphaLISA Acceptor beads is 20 µg/mL and AlphaScreen Streptavidin Donor beads is 20 µg/mL. The total assay volume is 25 µL. Volume of diluted reagents should be adjusted according to total number of assay points, plate format or assay volume.

### Steps for Preparing Reagents

1) Preparation of 1X AlphaLISA Immunoassay Buffer:

Add 1 mL of 10X AlphaLISA Immunoassay Buffer to 9 mL H<sub>2</sub>O.

2) Preparation of biotin human IgG Fab analyte standard dilutions:

- Reconstitute Biotin-Human IgG, Fab fragment to 2 mg/mL according to vendor's TDS, then further dilute with Milli-Q water to 30 µg/mL.
- Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

Tube	Vol. of hIgG Fab (µL)	Vol. of diluent (µL) *	[hIgG Fab] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of 30 µg/mL hIgG Fab	90	3.00E-06	3000 000
B	60 µL of tube A	120	1.00E-06	1000 000
C	60 µL of tube B	140	3.00E-07	300 000
D	60 µL of tube C	120	1.00E-07	100 000
E	60 µL of tube D	140	3.00E-08	30 000
F	60 µL of tube E	120	1.00E-08	10 000
G	60 µL of tube F	140	3.00E-9	3000
H	60 µL of tube G	120	1.00E-9	1000
I	60 µL of tube H	140	3.00E-10	300
J	60 µL of tube I	120	1.00E-10	100
K	60 µL of tube J	140	3.00E-11	30
L	60 µL of tube K	120	1.00E-11	10
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).

\*\* 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).

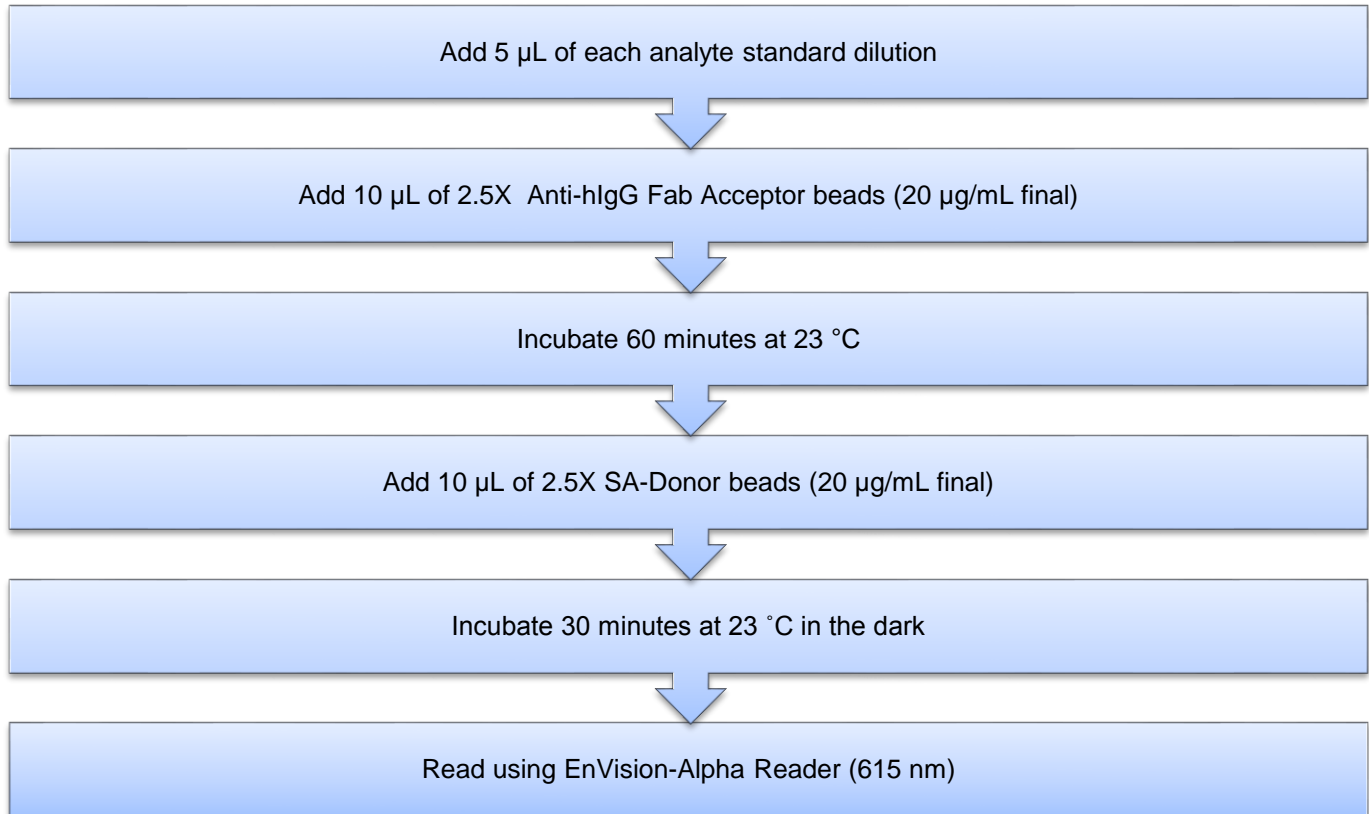
3) Preparation of 2.5X Anti-Human IgG Fab AlphaLISA Acceptor beads (50 µg/mL)

- Prepare just before use.
- Add 10 µL of 5 mg/mL Anti-Human IgG Fab AlphaLISA Acceptor beads to 990 µL of 1X AlphaLISA Immunoassay Buffer.

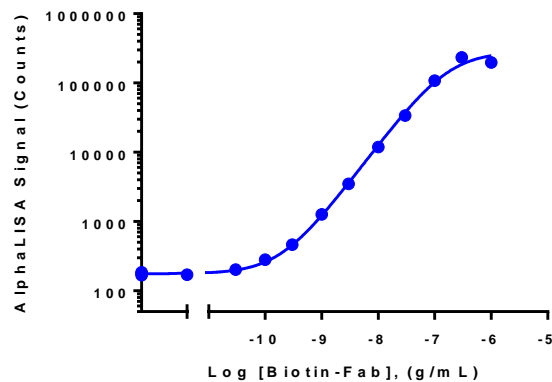
4) Preparation of 2.5X Streptavidin (SA) Donor beads (50 µg/mL):

- a. Prepare just before use and keep the beads under subdued laboratory lighting
- b. Add 10  $\mu\text{L}$  of 5 mg/mL Streptavidin (SA) Donor beads Donor beads to 990  $\mu\text{L}$  of 1X AlphaLISA Immunoassay Buffer.

5) In a white Optiplate (384 wells):



Typical results in 1X AlphaLISA Immunoassay Buffer:



The data was generated using a 25  $\mu\text{L}$  final volume in a white Optiplate-384 microplate and an EnVision-Alpha Reader 2102 with Alpha option.

## 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-washassays/alpha-troubleshooting.html>

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