CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaLISA®

Anti-Human IgG Fab Acceptor Beads

Product number: AL177C **Lot Number:** 3333781

Material provided: 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.

Product Format: AL177C: 250 µg, 50 µL, 500 assay points

AL177M: 5 mg, 1 mL, 10 000 assay points

AL177R: 25 mg, 5 mL, 50 000 assay points

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

Manufacturing date: July 12, 2024 **Document version:** 1

Product Information

Application: This product is intended for use in homogenous Alpha assays to capture human IgG Fab fragment. This product has minimum cross reactivity (<1%) with human IgG Fc, IgA, IgM and mouse, rat, rabbit, and goat IgG.

Storage: Store product in the dark at 4 ºC.

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.

Quality Control

Lot to lot consistency is confirmed in an Alpha assay. Maximum and minimum signals and EC50 were measured on the EnVision Multilabel Plate Reader with Alpha option. 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 assay quality

EC_{50}:	158.96 ng/mL
Min counts:	480 counts
Max counts:	178504 counts

Titration Assay (Quality Control Procedure)

This protocol provides a means to verify product performance. The following reagents and materials are recommended.

Item	Suggested source
White OptiPlate™-384	Revvity Inc.
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.
EnVision®-Alpha Reader	Revvity Inc.
AlphaScreen® Streptavidin coated Donor Beads	Revvity Inc.
Biotin-Human IgG,, Fab fragment	JAcksonImmuno, 009-060-007
AlphaLISA Immunoassay Buffer 10X	Revvity Inc.

Recommendations

- AlphaScreen® Donor beads are light-sensitive. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters) can be applied to light fixtures.
- Sodium azide should not be added to stock solutions or assay components. Final concentrations of sodium azide higher than 0.001 % will decrease the AlphaLISA signal.
- Spin down tubes briefly before use to improve recovery of content (2,000 x g, 10-15 sec). Resuspend all reagents by vortexing before use.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Adhesive Sealing Film to reduce evaporation during incubation. Microplates are read with the TopSeal-A Film on the plate.
- Total signal varies with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for all plates.
- 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, Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).

Protocol

1) Preparation of 1X AlphaLISA Immunoassay Buffer:
Add 1 mL of 10X AlphaLISA Immunoassay Buffer to 9 mL H_2O.

2) Preparation 1.7X hIgG Fab dilutions:
   a. 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.
   a. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

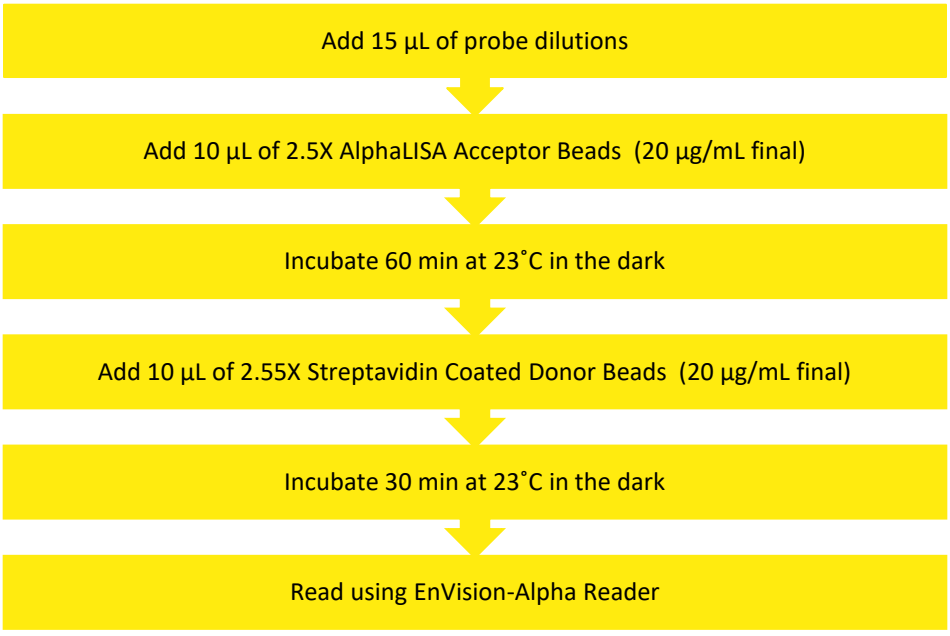
Tube	*Volume of* Probe	Volume of buffer (µL)	[hIgG Fab] (g/mL) in 15 µL (1.7X)	[hIgG Fab] (pg/mL) in 5 µL
A	10 µL of 30 µg/mL hIgG Fab	90	3.0E-6	3000 000

B	60 µL of tube A	120	1.0E-6	1000 000
C	60 µL of tube B	140	3.0E-7	300 000
D	60 µL of tube C	120	1.0E-7	100 000
E	60 µL of tube D	140	3.0E-8	30 000
F	60 µL of tube E	120	1.0E-8	10 000
G	60 µL of tube F	140	3.0E-9	3000
H	60 µL of tube G	120	1.0E-9	1000
I	60 µL of tube H	140	3.0E-10	300
J	60 µL of tube I	120	1.0E-10	100
K	60 µL of tube J	140	3.0E-11	30
L	60 µL of tube K	120	1.0E-11	10
M	0	100	0	0
N	0	100	0	0
O	0	100	0	0
P	0	100	0	0

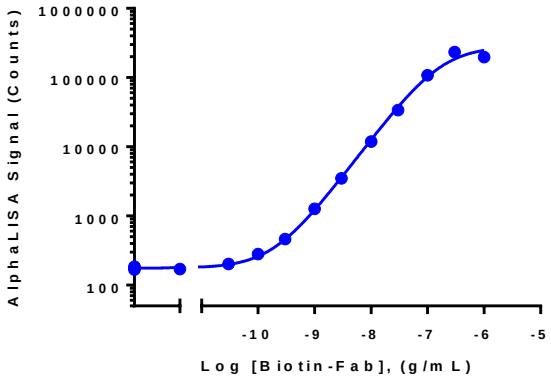
3) Preparation of 2.5X AlphaLISA Acceptor beads (50 µg/mL):)
Add 10 µL of 5 mg/mL AlphaLISA beads to 990 µL of 1X AlphaLISA Immunoassayl Assay Buffer.

4) Preparation of 2.5X Streptavidin-coated Donor Beads (50 µg/mL):
Keep the beads under subdued laboratory lighting. Add 10 µL of 5 mg/mL Streptavidin-coated Donor Beads to 990 µL of 1X AlphaLISA Immunassay Assay Buffer.

5) In a OptiPlate-384 microplate:



Typical Product Data



Please visit our website for additional information on AlphaLISA technology at www.revvity.com

AL177-R Rev01

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