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Anti-Human IgG Alpha Donor Beads ToolBox

Product number: AS114 D/M/R

Lot number: 2862011

Manufacturing date: APRIL 23, 2021

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Product Format

Catalog #	Size	Volume	Assay Points*
AS114D	1 mg	200 µL	2 000
AS114M	5 mg	1000 µL	10 000
ASL114R	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

Product Information

- Description:** Anti-hIgG Alpha Donor Beads at 5 mg/mL in PBS pH 7.4 supplemented with 0.05% Proclin-300 as a preservative.
- Application:** This product is intended for use in homogenous Alpha assays to capture human hIgG.
- Specificity:** This product does not cross react with human IgA, IgE, IgD, IgM and mouse IgG, rabbit IgG, swine IgG, and goat IgG.
- Storage:** Store in the dark 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 EC₅₀ 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: 35019 counts
Minimum Counts: 59 counts
EC₅₀: 0.02 nM

Titration Assay (Quality Control Protocol)

This protocol provides a means to verify product performance. The following reagents and materials are used, but not provided.

Items	Suggested Source	Catalog #
Anti-FITC AlphaLISA® Acceptor beads	PerkinElmer	AL127C (250 µg) AL127M (5 mg) AL127R (25 mg)
Fluorescein (FITC) ChromPure Human IgG, whole molecule	Jackson Immuno Research	009-090-003
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	-

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 H₂O 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

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BEFORE USE

This titration protocol is designed for 12 dilutions of the hIgG with triplicate determinations along with 12 background points. Final concentration of AlphaLISA Acceptor and Alpha Donor beads in the 25 μ L final assay volume is 20 μ g/mL. Volume of diluted reagents should be adjusted according to total number of assay points, plate format or assay volume.

- 1) Preparation of 1X AlphaLISA Immunoassay Buffer:
Add 1 mL of 10X AlphaLISA Immunoassay Buffer to 9 mL H₂O.
- 2) Preparation FITC-hIgG standard dilutions:
 - a. Thaw one vial of 500 nM FITC-hIgG (20 μ L). Vortex the tube briefly.
 - b. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

Tube	Vol. of FITC-hIgG (μ L)	Vol. of diluent (μ L) *	[FITC-hIgG] in standard curve	
			(M in 15 μ L)	(pM in 15 μ L)
A	3 μ L of 500 nM	147	1.0E-08	10000
B	60 μ L of tube A	140	3.0E-09	3000
C	60 μ L of tube B	120	1.0E-09	1000
D	60 μ L of tube C	140	3.0E-10	300
E	60 μ L of tube D	120	1.0E-10	100
F	60 μ L of tube E	140	3.0E-11	30
G	60 μ L of tube F	120	1.0E-11	10
H	60 μ L of tube G	140	3.0E-12	3
I	60 μ L of tube H	120	1.0E-12	1
J	60 μ L of tube I	140	3.0E-13	0.3
K	60 μ L of tube J	120	1.0E-13	0.1
L	60 μ L of tube K	140	3.0E-14	0.03
M ** (background)	0	100	0	0
M ** (background)	0	100	0	0
M ** (background)	0	100	0	0
M ** (background)	0	100	0	0

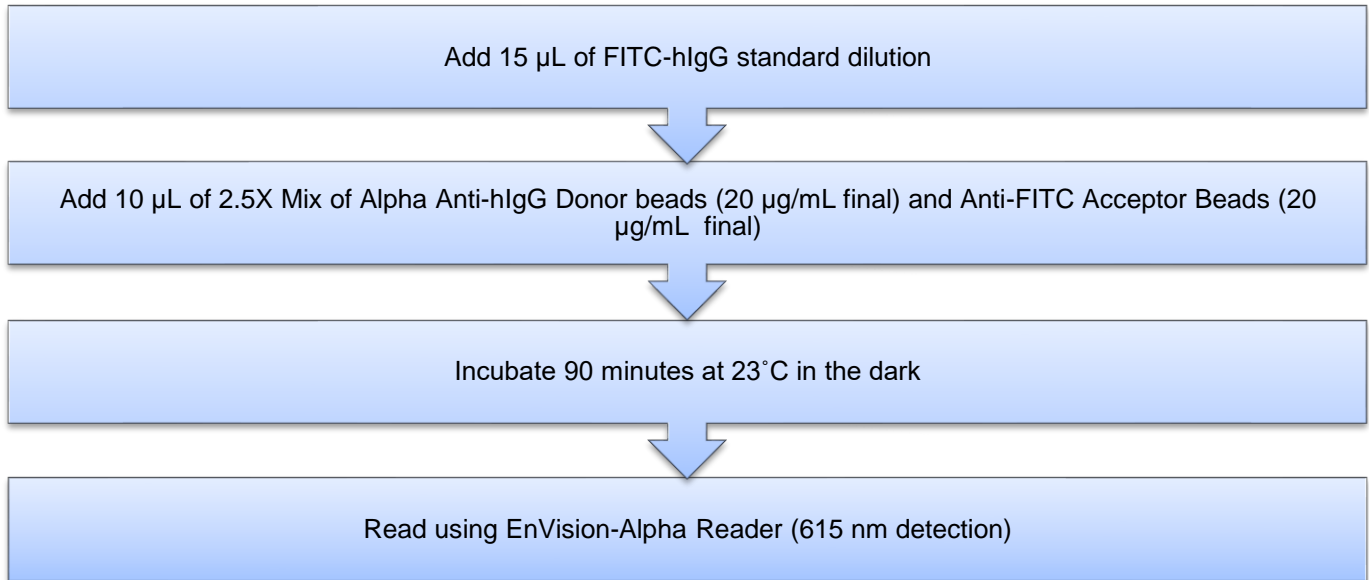
* Dilute standards in diluent (e.g. 1X AlphaLISA Immunoassay Buffer).
At low concentrations, a significant amount of probe can bind to the vial. Therefore, load the probe dilutions into the assay microplate within 60 minutes of preparation.

** For calculating background signal one background point in triplicate can be used (3 wells).

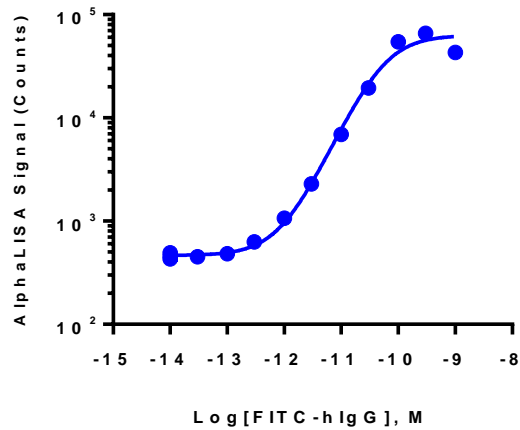
- 3) Preparation of 2.5X mix of Alpha Anti-hIgG Donor beads (50 μ g/mL) and Anti-FITC Acceptor beads (50 μ g/mL):

 - a. Prepare just before use and keep the beads under subdued laboratory lighting.
 - b. Add 10 μ L of 5 mg/mL Alpha Anti-hIgG Donor beads and 10 μ L of 5 mg/mL Anti-FITC Acceptor beads to 980 μ L of 1X AlphaLISA Immunoassay Buffer. Mix briefly.

4) In a white Optiplate (384 wells):



Typical Product Data



The data was generated using a white Optiplate-384 microplate and an EnVision-Alpha Reader 2105 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-wash-assays/alpha-troubleshooting.html>

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