TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Human IgG Fc Fragment AlphaLISA Acceptor Beads

Product number: AL174 C/M/R

Lot number: 2604409

Manufacturing date: July 16, 2019

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

Contents

Product Information

Description: Human IgG Fc fragment AlphaLISA Acceptor Beads at 5 mg/mL in PBS pH 7.2 supplemented with 0.05% Proclin-300 as a preservative. The Fc used is a native human IgG Fc fragment.

Application: This product is intended for use in homogenous Alpha assays to capture Fc gamma receptors.

Formats:

Catalog #	Size	Volume	Assay Points*
AL174C	250 µg	50 µL	312
AL174M	5 mg	1000 µL	6 250
AL174R	25 mg	5000 µL	31 250

* The number of assay points is based on an assay volume of 40 µL using a final bead concentration of 20 µg/mL in 96-well format

Storage: Store in the dark at 4 °C.

Stability: This product is stable for at least 12 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_{50} 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.

EC_{50}:	0.64 nM
LDL:	2.71 pM
LLOQ:	5.33 pM
Min Counts:	206
Max Counts:	1155768

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_2O (18 MΩ•cm) to dilute 10X AlphaLISA HiBlock 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 Alpha Donor beads:

Kit components	Suggested Source	Catalog #
AlphaLISA Streptavidin-coated Donor beads	PerkinElmer	6760002S (1 mg) 6760002 (5 mg) 6760002B (50 mg)
Human FCGR3A/ CD16a Protein (176 Val), Biotinylated	PerkinElmer	AL348S
1/2 Areaplate-96, White	PerkinElmer	6005560 (case of 50) 6005569 (case of 200)
TopSeal™-A Plus Adhesive Sealing Film	PerkinElmer	6050185
AlphaLISA HiBlock Buffer (10X)	PerkinElmer	AL004C(10 mL) AL004F(100 mL)
EnSpire® or EnVision® Multilabel Alpha Reader	PerkinElmer	-

Assay Protocol

1 Step Protocol – Dilution of standards can be done in 1X HiBlock Buffer. The protocol described below is for one standard curve (48 wells. *If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly*.

1) Preparation of 1X AlphaLISA HiBlock Buffer:
Add 1 mL of 10X AlphaLISA HiBlock Buffer to 9 mL Milli-Q H_2O.

2) Preparation of Human FCGR3A (176 Val) analyte standard dilutions:

   a. Reconstitute the 0.5 µg lyophilized human FCGR3A (176Val) in 100 µL Milli-Q H_2O to make 0.2 µM stock concentration. After reconstitution, store unused protein in -20 °C. Avoid multiple freeze/thaw cycles.

   b. Prepare standard dilutions as follows in 1X AlphaLISA HiBlock Buffer (change tips between each standard dilution):

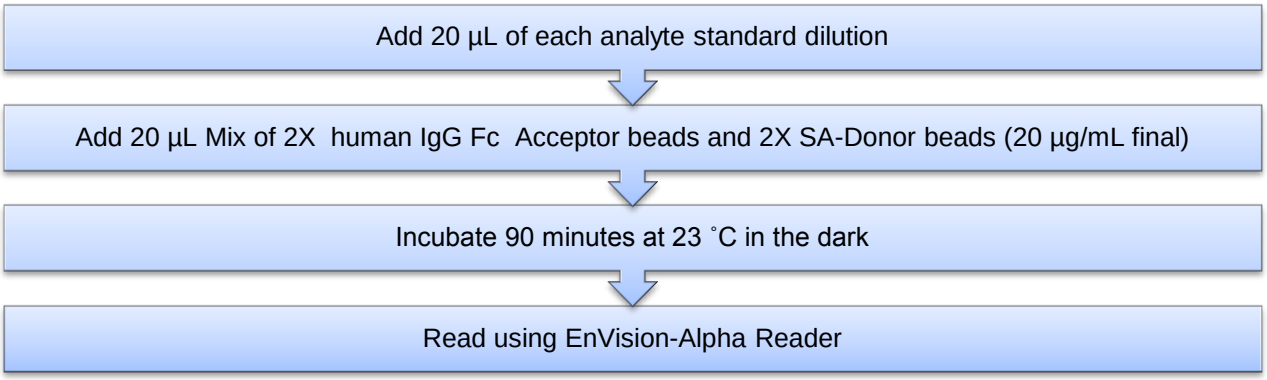
Tube	Vol. of hFCGR3A (176Val) (µL)	Vol. of diluent (µL) *	[hFCGR3A (176Val)] in standard curve	
			(2X, M)	(1X, M)
A	20 µL of reconstituted hFCGR3A (176Val)	180	2.00E-08	1.00E-08
B	60 µL of tube A	140	6.00E-09	3.00E-09
C	60 µL of tube B	120	2.00E-09	1.00E-09
D	60 µL of tube C	140	6.00E-10	3.00E-10
E	60 µL of tube D	120	2.00E-10	1.00E-10
F	60 µL of tube E	140	6.00E-11	3.00E-11
G	60 µL of tube F	120	2.00E-11	1.00E-11
H	60 µL of tube G	140	6.00E-12	3.00E-12
I	60 µL of tube H	120	2.00E-12	1.00E-12
J	60 µL of tube I	140	6.00E-13	3.00E-13
K	60 µL of tube J	120	2.00E-13	1.00E-13
L	60 µL of tube K	140	6.00E-14	3.00E-14
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 HiBlock 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).

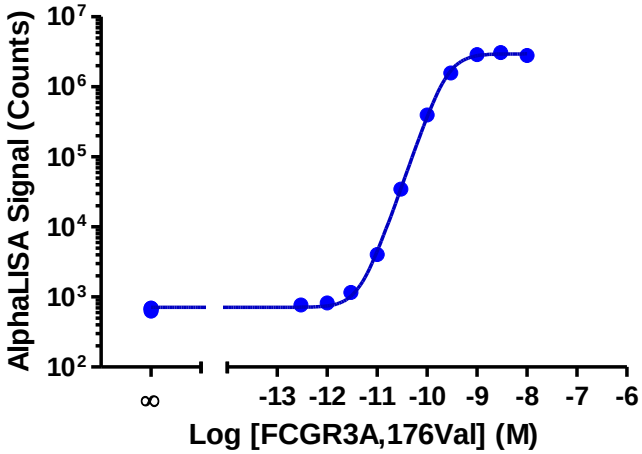
3) Preparation of the mix of 2X human IgG Fc full length Acceptor beads (40 µg/mL) and 2X Streptavidin (SA) Donor beads (40 µg/mL):

   a. Prepare just before use.
   b. Add 10 µL of 5 mg/mL AlphaLISA human IgG Fc Acceptor beads and 10 µL of 5 mg/mL Streptavidin (SA) Donor beads into 1230 µL of 1X AlphaLISA HiBlock Buffer.

4) In a half area 96 well plate:



Typical results in 1X AlphaLISA HiBlock Buffer:



The data was generated using a half area 96 well microplate (the background points are one row separated from sample points to avoid the signal crosstalk) and the plate was read by an EnVision-Alpha Reader 2103 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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