CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaScreen®

Human IgG Fc Fragment Donor Beads

Product number: AS113D **Lot Number:** 3307341

Material provided: Human IgG Fc fragment AlphaLISA Donor Beads at 5 mg/mL in PBS pH 7.2 supplemented with 0.05% Kathon as a preservative. The Fc used is a native human IgG Fc fragment.

Product Format: AS113D: 1 mg, 200µL, 625 assay points

AS113M: 5 mg, 1000µL, 3125 assay points

AS113R: 25 mg, 5000µL, 15625 assay points

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

Manufacturing date: March 20, 2024 **Document version:** 1

Product Information

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

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 EC_{50} 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}:	14.07 ng/mL
Min counts:	100 counts
Max counts:	33502 counts
Signal to Background:	335

Titration Assay (Quality Control Procedure)

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

Item	Suggested source
1/2 Areaplate-96, White	Revvity Inc.
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.
EnVision®-Alpha Reader	Revvity Inc.
AlphaLISA® Anti-6xHis Acceptor Beads	Revvity Inc.
AlphaLISA HiBlock Buffer 10X	Revvity Inc.
Human FCGR1/CD64 Protein (HisTag), Biotinylated	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.
- Use Milli-Q® grade water (18 MΩ•cm) to dilute the 10X HiBlock Buffer.
- 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 HiBlock Buffer:
Add 1 mL of 10X AlphaLISA HiBlock Buffer to 9 mL Milli-Q® grade H_2O.

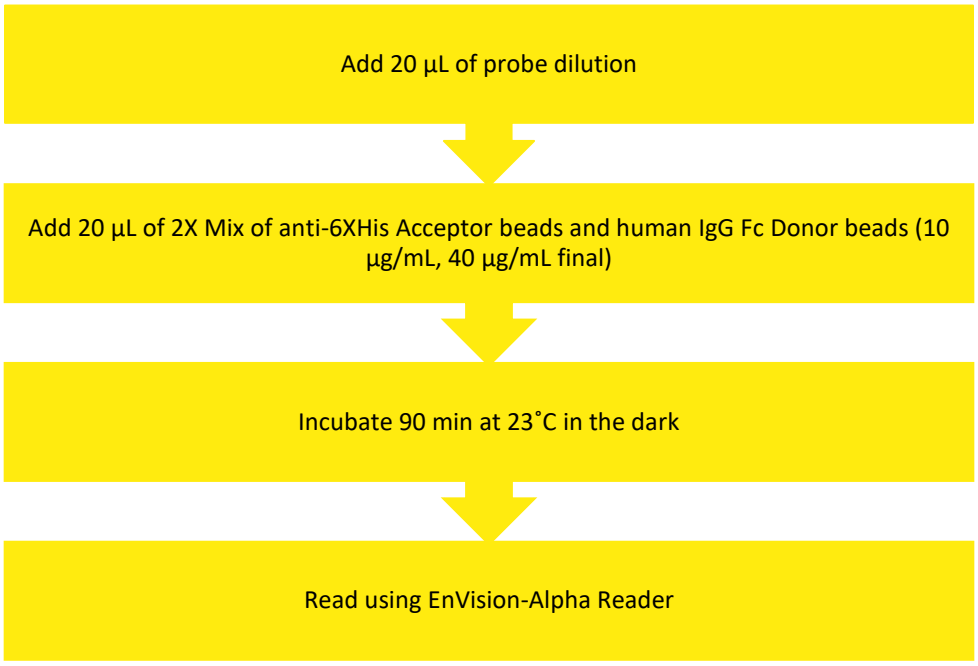
2) Preparation of Human FCGR1 dilutions:
Reconstitute the 1 µg lyophilized human FCGR1 in 100 µL Milli-Q H2O to make 10 µg/mL stock concentration. After reconstitution, store unused protein in -20 °C. Avoid multiple freeze/thaw cycles. Prepare dilution series in 1X AlphaLISA HiBlock Buffer as follows, changing the tip for each dilution:

Tube	Volume of Probe	Volume of buffer (µL)	[hFCGR1] (2X, g/mL)	[hFCGR1] (1X, g/mL) in final assay volume
A	12 µL of reconstituted hFCGR1	90	6.0E-7	3E-7
B	60 µL of tube A	70	2E-7	1E-7

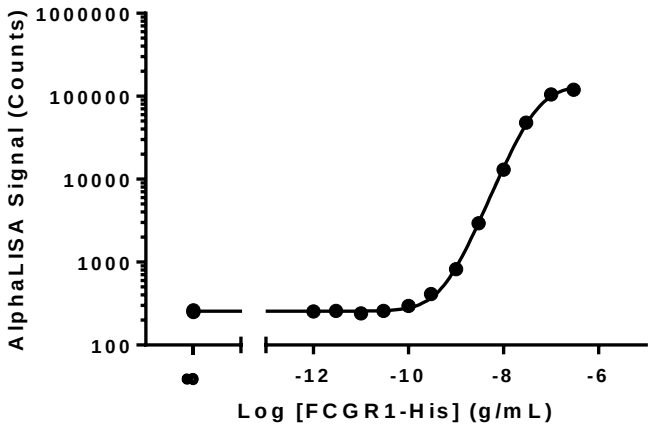
C	60 µL of tube B	60	6E-8	3E-8
D	60 µL of tube C	70	2E-8	1E-8
E	60 µL of tube D	60	6E-9	3E-9
F	60 µL of tube E	70	2E-9	1E-9
G	60 µL of tube F	60	6E-10	3E-10
H	60 µL of tube G	70	2E-10	1E-10
I	60 µL of tube H	60	6E-11	3E-11
J	60 µL of tube I	70	2E-11	1E-11
K	60 µL of tube J	60	6E-12	3E-12
L	60 µL of tube K	70	2E-12	1E-12
M	0	100	0	0
N	0	100	0	0
O	0	100	0	0
P	0	100	0	0

3) Preparation 2X mix of anti-6XHis AlphaLISA Acceptor beads (20 µg/mL) and human IgG Fc Donor beads (80 µg/mL):
   a. Prepare just before use.
   b. Add 10 µL of 5 mg/mL anti-6x His AlphaLISA Acceptor beads and 20 µL of 5 mg/mL human IgG Fc conjugated Donor beads into 1220 µL of 1X AlphaLISA HiBlock Buffer.

4) In a Half Area 96 well plate:



Typical Product Data



* The EC50 value was determined following a non-linear regression analysis using the sigmoidal dose-response curve model with variable slope. Only assay points up to the maximum signal were used for EC50 determination.

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

AS113-R Rev 01

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For a complete listing of our global offices, visit www.revvity.com