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CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaScreen®

Anti-FITC Donor Beads

Product number: AS115M **Lot Number:** 3256596

Material provided: Anti-FITC Alpha Donor Beads at 5 mg/mL in PBS pH 7.4 supplemented with 0.05% Kathon as a preservative.

Product Format: AS115D: 1 mg, 200µL, 2000 assay points

AS115M: 5 mg, 1000µL, 10 000 assay points

AS115R: 25 mg, 5000µL, 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: 1/24/2024 **Document version:** 1

Product Information

Application: This product is intended for use in homogenous Alpha assays to capture FITC labeled molecules.

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}:	0.23 nM
Min counts:	53 counts
Max counts:	9766 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.
AlphaLISA® Anti-hIgG Acceptor Beads	Revvity Inc.
FITC ChromPure Human IgG, whole molecule	Jackson ImmunoResearch (Cat # 009-090-003)
AlphaLISA Universal Buffer 5X	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 5X AlphaLISA Universal 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 Universal Buffer:
Add 2 mL of 5X AlphaLISA Universal Buffer to 8 mL Milli-Q® grade H_2O.

2) Preparation FITC-hIgG dilutions:
Thaw probe of 500 nM stock solution. Prepare dilution series in 1X AlphaLISA Universal Buffer as follows, changing the tip for each dilution:

Tube	Volume of Probe	Volume of buffer (µL)	FITC-hIgG (M) in 5 µL	FITC-hIgG (pM) in (5 µL)
A	6 µL of 50 nM	294	1E-8	10000
B	60 µL of tube A	140	3E-9	3000
C	60 µL of tube B	120	1E-9	1000
D	60 µL of tube C	140	3E-10	300
E	60 µL of tube D	120	1E-10	100
F	60 µL of tube E	140	3E-11	30
G	60 µL of tube F	120	1E-11	10
H	60 µL of tube G	140	3E-12	3
I	60 µL of tube H	120	1E-12	1
J	60 µL of tube I	140	3E-13	0.3
K	60 µL of tube J	120	1E-13	0.1
L	60 µL of tube J	140	3E-14	0.03
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 mix of Alpha Anti-FITC Donor Beads (50 µg/mL) and Anti-hIgG acceptor (50 µg/mL):
   a. Prepare just before use and keep the beads under subdued laboratory lighting.
   b. Add 10 µL of 5 mg/mL Alpha Anti-FITC Donor beads and 10 µL of 5 mg/mL Anti-hIgG Acceptor beads to 980 µL of 1X AlphaLISA Universal Buffer. Mix briefly.

4) In a OptiPlate-384 microplate:

Add 15 µL of probe dilution

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Add 10 µL of 2.5X Mix of Alpha Anti-FITC Donor beads (20 µg/mL final) and Anti-hIgG Acceptor Beads (20 µg/mL final)

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Incubate 90 min at 23˚C in the dark

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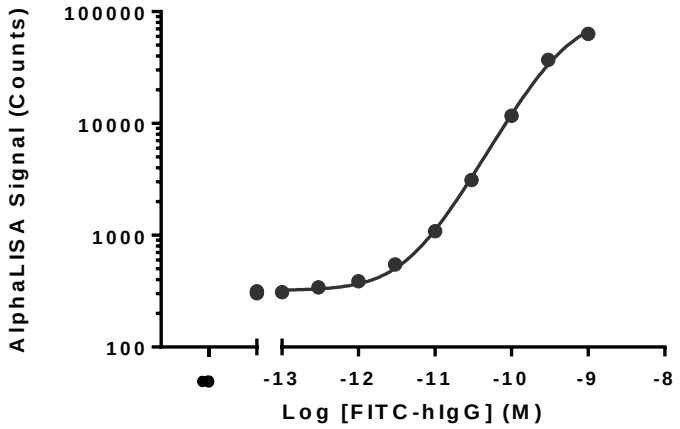
Read using EnVision-Alpha Reader

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

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