

AlphaScreen®

Protein A Donor Beads

Product number: AS102M **Lot Number:** 3245529

Material provided: Protein A Alpha Donor beads at 5 mg/mL in PBS pH 7.2 with 0.05% Kathon as a preservative. The protein used is a native protein.

Product Format: AS102D: 1 mg, 200µL, 2000 assay points
AS102M: 5 mg, 1000µL, 10 000 assay points
AS102R: 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: 12/20/2023 **Document version:** 1

Product Information

Application: This product is intended for use in homogeneous AlphaLISA assays to capture Fc region of IgG of several species (human IgG, human IgM, mouse IgG and rabbit IgG). AlphaLISA Acceptor beads must be ordered separately.

Storage: Store product in the dark at 4 °C.

Stability: This kit is stable for at least 12 months from the date of manufacture when stored in its original packaging and the recommended storage conditions. Diluted beads are light-sensitive. Therefore, beads must be used immediately after dilution.

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.

EC₅₀: 0.04 nM
Min counts: 88 counts
Max counts: 55423 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® Streptavidin Acceptor Beads	Revvity Inc.
Biotinylated Rabbit IgG	Jackson ImmunoResearch (Cat #011-060-003)
AlphaLISA Universal Assay 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.
- 1X AlphaLISA Universal Assay Buffer contains PBS, pH 7.5, 0.1% BSA, 0.01% Kathon. 1X AlphaLISA Universal Assay Buffer is used in the titration assay described below (Quality Control Protocol). Optimization of this assay buffer might be necessary in other assay types.
- 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 H₂O.
- 2) Preparation 1.7X (Biotinylated Rabbit IgG) dilutions:
Dilute probe to a 50 nM stock solution. Prepare dilution series in 1X AlphaLISA Universal Assay Buffer as follows, changing the tip for each dilution:

Tube	Volume of probe	Volume of buffer (µL)	Biotinylated Rabbit IgG (M) in 15 µL (1.7X)	Biotinylated Rabbit IgG (M) in final assay volume (25 µL)
A	5 µL of 50 nM	141	1.7E-9	1.0E-9
B	60 µL of tube A	140	5.1E-10	3.0E-10
C	60 µL of tube B	120	1.7E-10	1.0E-10

D	60 μ L of tube C	140	5.1E-11	3.0E-11
E	60 μ L of tube D	120	1.7E-11	1.0E-11
F	60 μ L of tube E	140	5.1E-12	3.0E-12
G	60 μ L of tube F	120	1.7E-12	1.0E-12
H	60 μ L of tube G	140	5.1E-13	3.0E-13
I	60 μ L of tube H	120	1.7E-13	1.0E-13
J	60 μ L of tube I	140	5.1E-14	3.0E-14
K	60 μ L of tube J	120	1.7E-14	1.0E-14
L	0	140	0	0

3) Preparation of 5X Alpha Donor beads (100 μ g/mL):

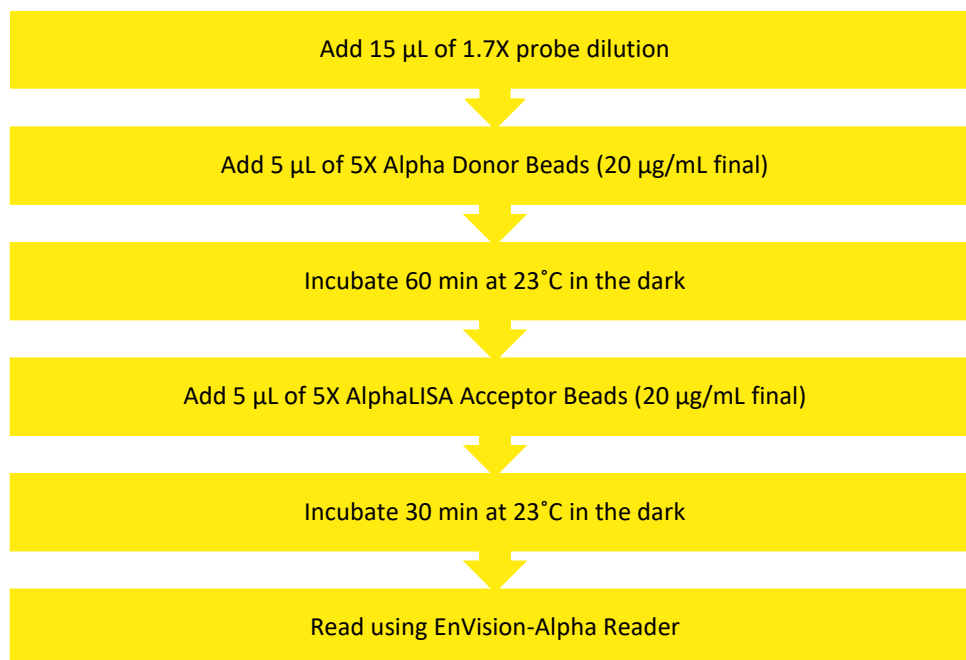
Diluted beads are light-sensitive. Therefore, beads must be used immediately after dilution.

Add 5 μ L of 5 mg/mL Alpha Donor beads to 245 μ L of 1X AlphaLISA Universal Assay Buffer.

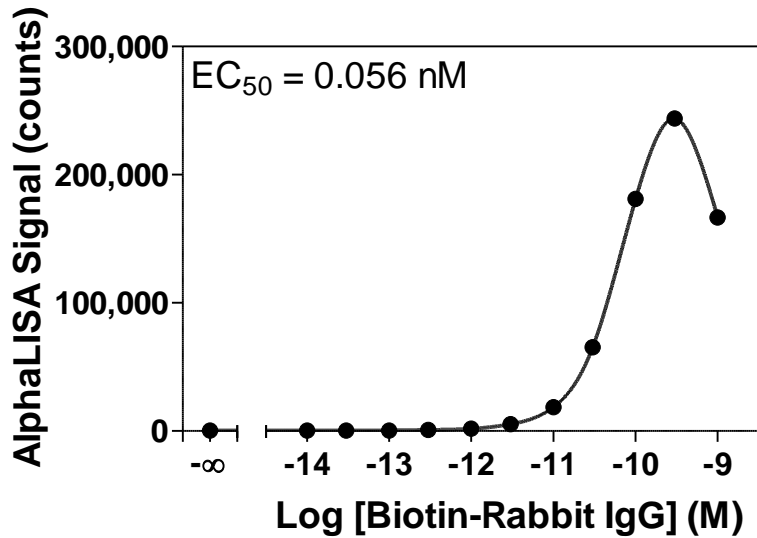
4) Preparation of 5X AlphaLISA Acceptor Beads (100 μ g/mL):

Add 5 μ L of 5 mg/mL AlphaLISA Acceptor beads to 245 μ L of 1X AlphaLISA Universal Assay Buffer.

5) In a OptiPlate-384 microplate:



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.revivity.com

The information provided in this document is valid for the specified lot number and date of analysis. This information is for reference purposes only and does not constitute a warranty or guarantee of the product's suitability for any specific use. Revvity, Inc., its subsidiaries, and/or affiliates (collectively, "Revvity") do not assume any liability for any errors or damages arising from the use of this document or the product described herein. REVVITY EXPRESSLY DISCLAIMS ALL WARRANTIES, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, REGARDLESS OF WHETHER ORAL OR WRITTEN, EXPRESS OR IMPLIED, ALLEGEDLY ARISING FROM ANY USAGE OF ANY TRADE OR ANY COURSE OF DEALING, IN CONNECTION WITH THE USE OF INFORMATION CONTAINED HEREIN OR THE PRODUCT ITSELF.

AS102-R Rev 01

revvity

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000 www.revivity.com

For a complete listing of our global offices, visit www.revivity.com
Copyright ©2023, Revvity, Inc. All rights reserved.