TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

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

Strep-Tactin® Alpha Donor Beads

Product No.: **AS106D (1 mg)**
AS106M (5 mg)
AS106R (25 mg)

Lot No.: P1

Product Formats

Catalog #	Size	Volume	Assay Points*
AS106D	1 mg	200 µL	2 000
AS106M	5 mg	1 mL	10 000
AS106R	25 mg	5 mL	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.

Manufacturing Date: March 08, 2011

Product Information

Description: *Strep*-Tactin® Alpha Donor beads at 5 mg/mL in PBS pH 7.2 with 0.05% Proclin-300 as a preservative. The protein used is a recombinant protein. *Strep*-Tactin® is manufactured by IBA and the related patents are held by IBA. PerkinElmer has the non-exclusive right to coat AlphaLISA and Alpha beads with *Strep*-Tactin®.

Application: This product is intended for use in homogeneous AlphaLISA assays to capture *Strep*-tag® II, One-STrEP-tag, or biotin-tagged targets. AlphaLISA Acceptor beads must be ordered separately.

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.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Quality Control

Lot-to-lot consistency is confirmed by a Quality Control AlphaLISA titration assay read on an EnVision® HTS Alpha instrument (see protocol below). We certify that the results meet our quality release criteria. *Note: maximum counts will vary depending on assay conditions as well as between lots. This variation has no impact on assay quality.*

Maximum signal: 43 684 counts
Minimum signal: 214 counts
EC_{50}: 3.15 nM

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Titration Assay (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:

Item	Suggested Source	Catalog #
Protein A AlphaLISA Acceptor beads	PerkinElmer	AL101C (250 µg) AL101M (5 mg) AL101R (25 mg)
Biotinylated Rabbit IgG	Jackson ImmunoResearch	011-060-003
AlphaLISA Universal Assay Buffer, 5X	PerkinElmer	AL001C (10 mL) AL001F (100 mL)
White OptiPlate™-384	PerkinElmer	6007290
TopSeal™-A Adhesive Sealing Film	PerkinElmer	6005185
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 prewet the tip.
- Alpha Donor beads are light-sensitive. All steps using the Alpha Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (Roscolux filters #389 from Rosco, or the equivalent) can be applied to light fixtures. All the other assay reagents can be used under normal light conditions.
- Sodium azide should not be added to stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal.
- Centrifuge the 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 Assay Buffer.
- When diluting the probe, change tips after each dilution. When loading reagents in the assay microplate, change tips after each reagent addition and between each set of reagents.
- When reagents are added in the microplate, make sure the liquids are at the bottom of the well.
- 1X AlphaLISA Universal Assay Buffer contains PBS, pH 7.5, 0.1% BSA, 0.01% Proclin-300. This buffer is used in the titration assay described below (Quality Control Protocol). Optimization of the 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 Alpha-enabled EnSpire or EnVision Multilabel Reader 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%).

Quality Control Protocol

This titration protocol is designed for 12 dilutions of the probe with triplicate determinations. Final concentration of AlphaLISA Acceptor and Alpha Donor beads in the 25 µL final assay volume is 20 µg/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 Universal Assay Buffer:
Add 1 mL of 5X AlphaLISA Universal Assay Buffer to 4 mL H_2O.

2) Preparation of 1.7X probe (Biotinylated Rabbit IgG) dilutions:
Dilute probe to a 1.25 µM stock solution.
Prepare dilution series in 1X AlphaLISA Universal Assay Buffer as follows, changing tip for each dilution:

Tube	Volume of Probe	Volume of 1X Buffer (µL)	[Biotinylated Rabbit IgG] (M)	
			in 15 µL (1.7X)	in 25 µL Final Assay Volume
A	20 µL of 1.25 µM	127	1.7E-07	1.0E-07
B	60 µL of tube A	140	5.1E-08	3.0E-08
C	60 µL of tube B	120	1.7E-08	1.0E-08
D	60 µL of tube C	140	5.1E-09	3.0E-09
E	60 µL of tube D	120	1.7E-09	1.0E-09
F	60 µL of tube E	140	5.1E-10	3.0E-10
G	60 µL of tube F	120	1.7E-10	1.0E-10
H	60 µL of tube G	140	5.1E-11	3.0E-11
I	60 µL of tube H	120	1.7E-11	1.0E-11
J	60 µL of tube I	140	5.1E-12	3.0E-12
K	60 µL of tube J	120	1.7E-12	1.0E-12
L	0	140	0	0

3) Preparation of 5X Alpha Donor beads (100 µg/mL):
Keep the beads under subdued laboratory lighting.
Add 5 µL of 5 mg/mL Alpha Donor beads to 245 µL of 1X AlphaLISA Universal Assay Buffer.

4) Preparation of 5X AlphaLISA Acceptor beads (100 µg/mL):
Add 5 µL of 5 mg/mL AlphaLISA Acceptor beads to 245 µL of 1X AlphaLISA Universal Assay Buffer.

5) In a white opaque OptiPlate-384 microplate:

Add 15 µL of 1.7X probe dilution

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Add 5 µL of 5X Alpha Donor beads (20 µg/mL final)

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Incubate 60 min at 23ºC in the dark

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Add 5 µL of 5X AlphaLISA Acceptor beads (20 µg/mL final)

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Incubate 30 min at 23ºC in the dark

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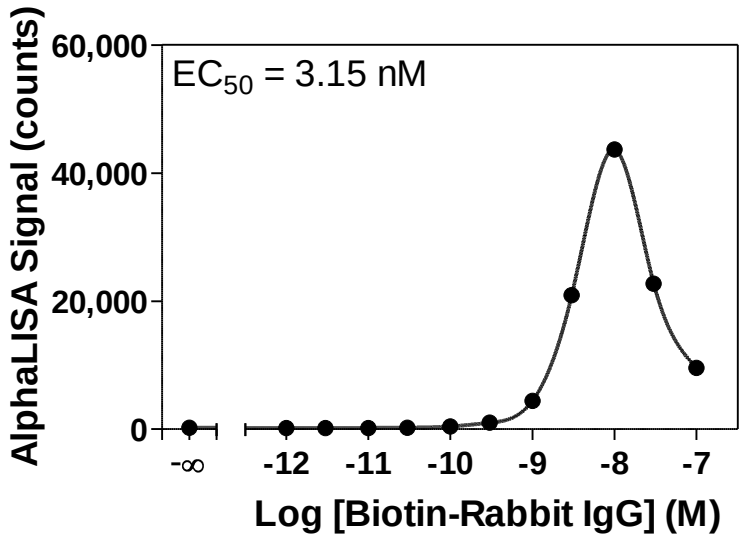
Read using EnSpire or EnVision Multilabel Alpha Reader

Typical Product Data



Titration assay using the Quality Control protocol. Signal was detected with an EnVision Alpha instrument 2102.

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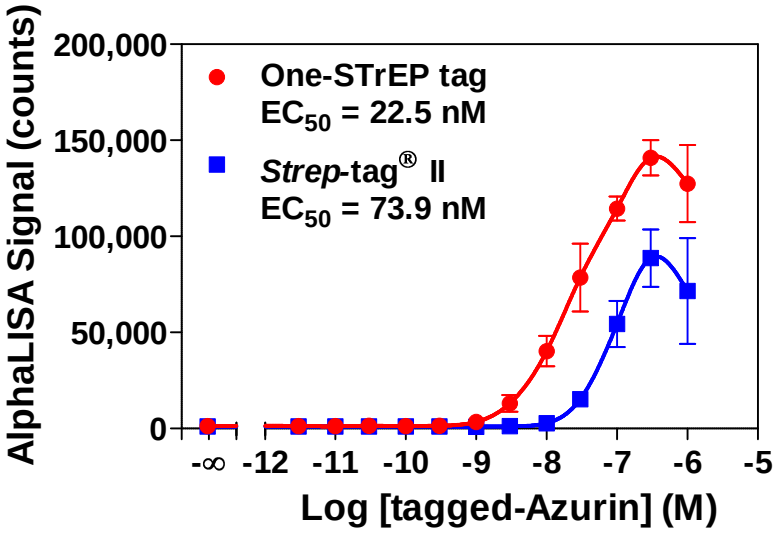
Additional Data

Depending on the assay components, order of addition of the bead reagents may need optimization.



Titration assay using the Quality Control protocol. Signal was detected with an EnVision Alpha instrument 2101.

Please visit our website for additional information on the AlphaLISA technology at www.perkinelmer.com/AlphaTech.

This product is not for resale or distribution except by authorized distributors.

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