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

Anti-FLAG® Acceptor Beads

Product No.: **AL112C (250 µg)**
AL112M (5 mg)
AL112R (25 mg)

Lot No.: 677-686-A

Material Provided

Formats:

Catalog number	Size	Volume	Assay points
AL112C	250 µg	50 µL	500
AL112M	5 mg	1 mL	10 000
AL112R	25 mg	5 mL	50 000

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: July 05, 2011

Description: AlphaLISA Anti-FLAG® Acceptor Beads at 5 mg/mL in PBS pH 7.2 supplemented with 0.05% Proclin-300 as a preservative.

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.

Product Information

Intended use: This product is intended for use in homogeneous AlphaLISA assays for the capture of FLAG®-tagged targets.

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

Quality Control

Lot-to-lot consistency is confirmed by a Quality Control AlphaScreen® 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 589 408 counts
Minimum signal: 371 counts
EC_{50}: 30.37 nM

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Titration Assay (Quality Control Protocol)

This protocol provides a means to verify product performance.

The following reagents and materials are recommended.

Item	Suggested Source	Catalog #
AlphaScreen® Streptavidin-coated Donor Beads	PerkinElmer LAS, Inc.	6760002S (1 mg) 6760002 (5 mg) 6760002B (50 mg)
Biotin-Chromalink-DYKDDDDK amide (Biotin-FLAG®)	SoluLink Biosciences	Custom
AlphaLISA Universal Assay Buffer, 5X	PerkinElmer LAS, Inc.	AL001C (10 mL) AL001F (100 mL)
White OptiPlate™-384	PerkinElmer LAS, Inc.	6007290
TopSeal™-A Adhesive Sealing Film	PerkinElmer LAS, Inc.	6005185
EnVison® Multilabel Reader with the Alpha Option	PerkinElmer LAS, Inc.	-

Recommendations:

- AlphaScreen® Donor beads are light-sensitive. All AlphaLISA assays using the Streptavidin-coated Donor Beads should be performed under subdued laboratory lighting of less than 100 lux. Alternatively, green filters (Roscolux filters #389 from Rosco, or the equivalent) can be applied to light fixtures. Incubation with AlphaScreen® Donor beads should always be performed in the dark. For example, assay reactions in a microplate can be covered with an opaque microplate.
- 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% Proclin-300. 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:

This protocol is recommended for generating one titration curve in a 25 µL final assay volume (12 concentrations, triplicate determinations with manual pipetting). If more assay points are needed, volumes should be adjusted accordingly.

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 Biotin-FLAG® IgG dilutions:

   Prepare 1.7X dilutions in 1X AlphaLISA Universal Assay Buffer as follows:

Tube	Volume of Biotin-FLAG®	Volume of buffer (µL)	[Biotin-FLAG®] (M) in 15 µL (1.7X)	[Biotin-FLAG®] (M) in 25 µL final assay volume
A	5 µL of 100 µM	289	1.7E-6	1.0E-6
B	60 µL of tube A	140	5.1E-7	3.0E-7
C	60 µL of tube B	120	1.7E-7	1.0E-7
D	60 µL of tube C	140	5.1E-8	3.0E-8
E	60 µL of tube D	120	1.7E-8	1.0E-8
F	60 µL of tube E	140	5.1E-9	3.0E-9
G	60 µL of tube F	120	1.7E-9	1.0E-9
H	60 µL of tube G	140	5.1E-10	3.0E-10
I	60 µL of tube H	120	1.7E-10	1.0E-10
J	60 µL of tube I	140	5.1E-11	3.0E-11
K	60 µL of tube J	120	1.7E-11	1.0E-11
L	0	140	0	0

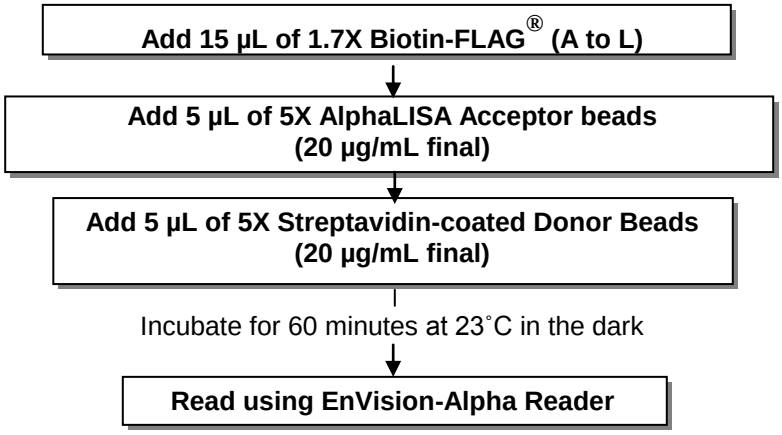
3) Preparation of 5X AlphaLISA Acceptor beads (100 µg/mL):

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

4) Preparation of 5X Streptavidin-coated Donor Beads (100 µg/mL):

   Keep the beads under subdued laboratory lighting.
   Add 5 µL of 5 mg/mL Streptavidin-coated Donor Beads to 245 µL of 1X AlphaLISA Universal Assay Buffer.

5) In an OptiPlate-384 microplate:



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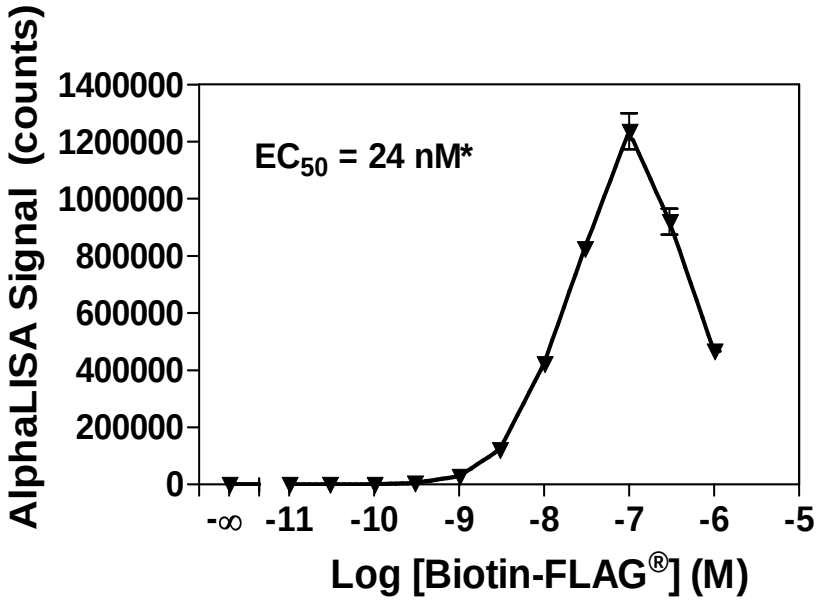
Typical Product Data



* The EC_{50} 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 EC_{50} determination (in this case, up to 100 nM).

Suggested Materials and Instrumentation

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www.perkinelmer.com/AlphaTech

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