Technical Data
Certificate of Analysis

Caution: For Laboratory Use. Research chemicals for research purposes only.

AlphaScreen® Nickel Chelate Donor Beads

PRODUCT NUMBERS: **AS101D (1 mg)**
AS101M (5 mg)
AS101R (25 mg)

LOT NO.: XXX-XXX-X

MATERIAL PROVIDED

DESCRIPTION: AlphaScreen Nickel Chelate Donor Beads at 5 mg/mL in 25 mM Hepes, pH 7.4, 100 mM NaCl supplemented with 0.05% Proclin-300 as a preservative.

FORMATS:

Catalog number	Size	Volume	Assay points
AS101D	1 mg	200 µL	2 000
AS101M	5 mg	1 mL	10 000
AS101R	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.

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 and the recommended storage conditions.

MANUFACTURING DATE: Month, day, year

PRODUCT INFORMATION

- This product is intended for use in homogeneous AlphaScreen or AlphaLISA assays for the capture of 6XHis-tagged targets.
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

QUALITY CONTROL

- This product is controlled for accurate bead concentration (5 mg/mL).
- Lot-to-lot consistency is confirmed by a Quality Control titration assay read on an EnVision-Alpha 2102 (see protocol below) to meet defined specifications.

PARAMETER	RESULTS	SPECIFICATIONS
Minimum signal:	XX counts	≤ 2 400 counts
EC_{50}:	XX nM	33 – 330 nM

There are no specifications on maximum signal. Maximum counts will vary depending on assay conditions as well as between lots. This variation has no impact on assay quality. For information, maximum signal obtained using this lot and the Quality Control titration assay was XXX counts.

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TITRATION ASSAY (QUALITY CONTROL PROTOCOL)

This protocol provides a means to verify product performance.

SPECIFIC REQUIRED REAGENTS AND MATERIALS

The following reagents and materials are recommended.

Item	Suggested Source	Catalog #
AlphaLISA® Glutathione Acceptor Beads	PerkinElmer LAS, Inc.	AL109C (250 µg) AL109M (5 mg) AL109R (25 mg)
GST, 6 His-tagged protein	Millipore Corp.	12-523
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 AlphaScreen or AlphaLISA assays using the Nickel Chelate 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 GST, 6 His-tagged dilutions:

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

Tube	Volume of GST, 6 His-tagged	Volume of buffer (µL)	[GST, 6 His-tagged] (M) in 15 µL (1.7X)	[GST, 6 His-tagged] (M) in final assay volume (25 µL)
A	7 µL of 37 µM	145	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

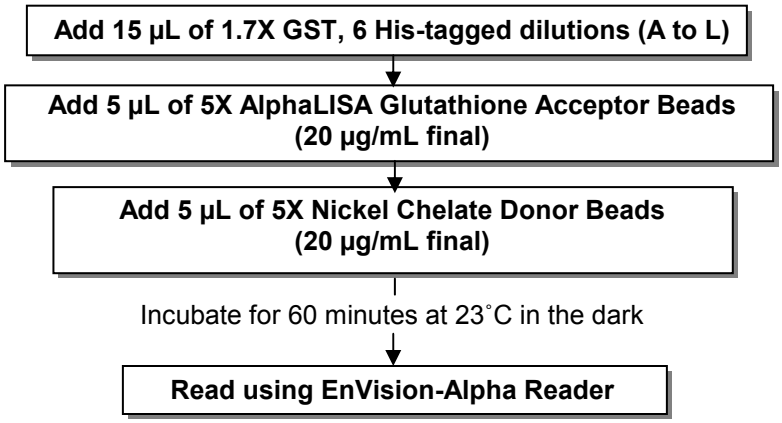
3) Preparation of 5X AlphaLISA Acceptor beads (100 µg/mL):

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

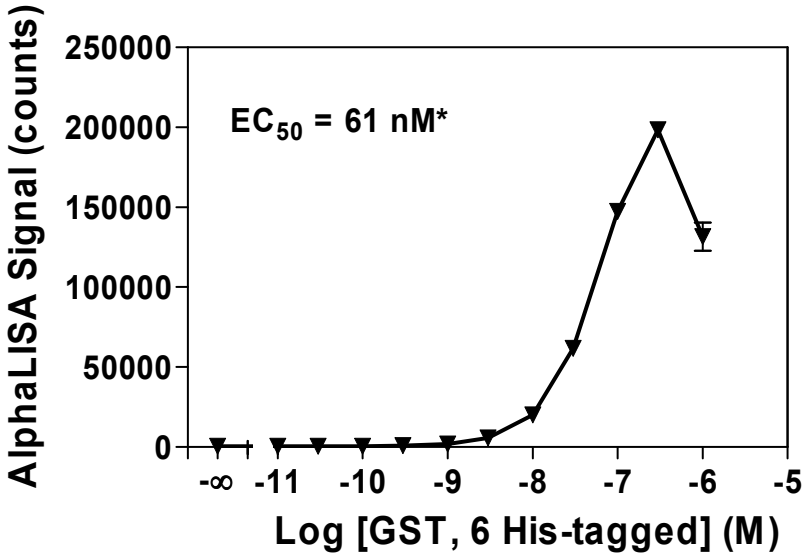
4) Preparation of 5X Nickel Chelate Donor Beads (100 µg/mL):

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

5) In an OptiPlate-384 microplate:



TYPICAL RESULT



* 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 300 nM).