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CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaScreen™

Anti-GST Donor Beads

Product number:	AS119D	**Lot Number:**	3387968

Material provided: Alpha Anti-GST Donor Beads at 5 mg/mL in PBS 1X pH 7.4 supplemented with 0.05% Kathon as a preservative

Product Format: AS119D: 1 mg, 200 µL, 2000 assay points

AS119M: 5 mg, 1000 µL, 10 000 assay points

AS119R: 25 mg, 5000 µL, 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:	1/31/2025	**Document version:**	1

Product Information

Application: This product is intended for use in homogeneous Alpha assays for the capture of GST.

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 signal, minimum signal, S/B 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}:	4.04 nM
Min counts:	353 counts
Max counts:	61,361 counts
S/B:	174

Titration Assay (Quality Control Procedure)

This protocol provides a means to verify product performance. The following reagents and materials are recommended.

Item	Suggested source
AlphaPlate™-384 light gray	Revvity Inc.
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.
EnVision™-Alpha Reader	Revvity Inc.
AlphaLISA Anti-6xHis Acceptor Beads	Revvity Inc.
GST, 6xHis-tagged peptide	Sigma Aldrich (Cat #12-523)
AlphaLISA PPI Assay Buffer 5X	Revvity Inc.

Recommendations

- Alpha 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 PPI Buffer.
- 1X AlphaLISA PPI 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 PPI Buffer:
Add 2 mL of 5X AlphaLISA PPI Buffer to 8 mL Milli-Q® grade H_2O.

2) Preparation 1.67X GST, 6xHis-tagged peptide dilutions:

Prepare a pre-dilution of GST, 6xHis-tagged peptide: add 10 µL of GST, 6xHis-tagged peptide stock solution (at 37 µM) to 90 µL of 1X AlphaLISA PPI Assay Buffer

Prepare 1.67X dilutions in 1X AlphaLISA PPI Assay Buffer as follows:

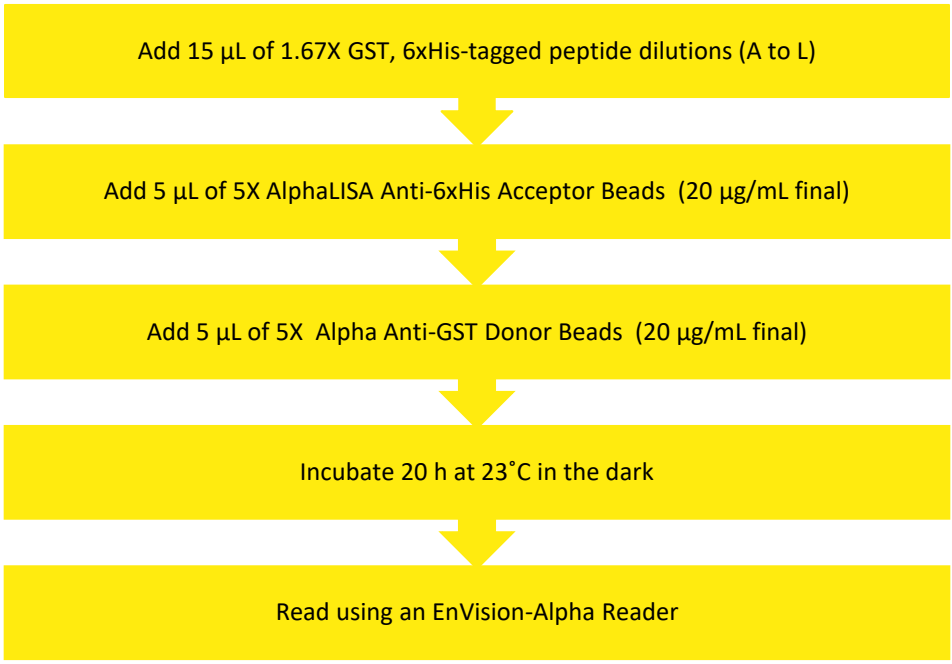
Tube	Volume of GST, 6xHis-tagged	Volume of buffer (µL)	GST, 6xHis-tagged] (M) in 15 µL (1.67X)	[GST, 6xHis-tagged] (M) in final assay volume (25 µL)
A	10 µL of pre-dilution	730	5.0E-8	3.0E-8
B	60 µL of tube A	120	1.67E-8	1.0E-8

C	60 µL of tube B	140	5.0E-9	3.0E-9
D	60 µL of tube C	120	1.67E-9	1.0E-9
E	60 µL of tube D	140	5.0E-10	3.0E-10
F	60 µL of tube E	120	1.67E-10	1.0E-10
G	60 µL of tube F	140	5.0E-11	3.0E-11
H	60 µL of tube G	120	1.67E-11	1.0E-11
I	60 µL of tube H	140	5.E-12	3.0E-12
J	60 µL of tube I	120	1.67E-12	1.0E-12
K	60 µL of tube J	140	5.0E-13	3.0E-13
L	0	140	0	0

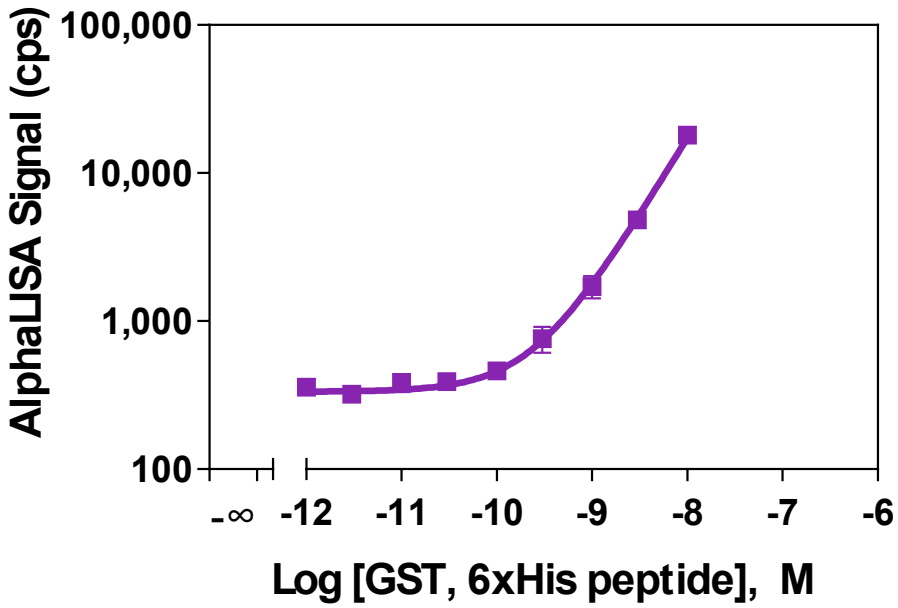
3) Preparation of 5X AlphaLISA Anti-6xHis Acceptor Beads (100 µg/mL):
Add 10 µL of 5 mg/mL AlphaLISA Anti-6xHis Acceptor Beads to 490 µL of 1X AlphaLISA PPI Assay Buffer.

4) Preparation of 5X Alpha Anti-GST Donor Beads (100 µg/mL):
Keep the beads under subdued laboratory lighting. Add 10 µL of 5 mg/mL Alpha Anti-GST Donor Beads to 490 µL of 1X AlphaLISA PPI Assay Buffer.

5) In a AlphaPlate-384 light gray microplate:



Typical **Product Data**



The signal was measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in the quality control procedure.

EC_{50} is calculated by Graphpad Prism with a "log(agonist) vs. response -- Variable slope (four parameters)" fitting. Only assay points up to the maximum signal were used for EC50 determination (in this case, up to 300 nM, corresponding to Tube C).
S/B is calculated on Tube C to ensure operating in the dynamic range of the assay.

Please visit our website for additional information on AlphaLISA technology at www.revvity.com

Revvity, Inc. (800) 762-4000 www.revvity.com

For a complete listing of our global offices, visit www.revvity.com