AlphaPlex™-645 Streptavidin Acceptor Beads

Product number: AP125SM-C

Lot number: 3180238

Manufacturing date: July 12, 2023

Research Use Only. Not for use in diagnostic procedures.

Product Information

Description: Streptavidin AlphaPlex-645 Protein A Acceptor Beads at 5 mg/mL in PBS pH 7.2 supplemented with 0.05% Kathon as a preservative. The protein used is a pure homogeneous preparation obtained from the culture broth of the bacterium *Streptomyces avidinii.*

Application: This product is designed for use as a tool to generate Alpha assays involving antibodies specifically without the need of conjugation to acceptor beads.

Formats:

Catalog #	Size	Volume	Assay Points
AP125Sm-C	250 µg	60 µL	500
AP125Sm-M	5 mg	1050 µL	10 000
AP125Sm-R	25 mg	5100 µL	50 000

The number of assay points is based on an assay volume of 25 µL in384-well assay plates using a final bead concentration of 20 µg/mL.

Storage: Store kit in the dark at +4˚C.

Stability: This product is stable for at least 4 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Sensitivity: EC_{50}: 0.1 nM

Hook point: M

Minimal signal: 1500 counts*

Maximal signal: 475000 counts*

*As determined on an EnVision® Multilabel Plate Reader with Alpha option 2104.

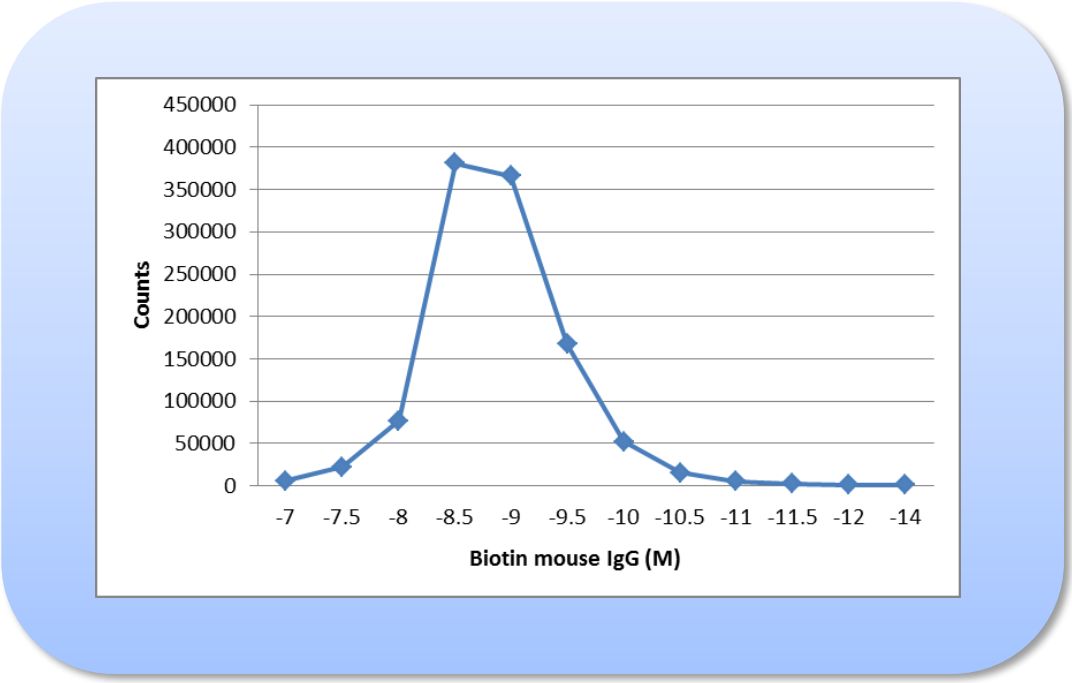


Figure. 1. Typical assay curve. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader with Alpha option 2104. The curve was obtained by mixing acceptor and donor beads with increasing concentrations of biotinylated mIgG. The EC_{50} was measured from the curve portion ranging from 0 analyte to the hook point..

Quality Control

Lot to lot consistency is confirmed in an Alpha assay. Maximum signals were measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in this technical data sheet. 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}:	0.29 nM
Minimal signal:	70 counts
Maximal signal:	183743 counts

Quality Control Protocol

STREPTAVIDIN protocol (STREPTAVIDIN incubation steps) – Dilution of standards in 1X PBS + 0.1% Tween 20

The protocol described below is recommended when generating one standard curve in triplicate with a 25 µL final assay volume (48 wells, triplicate determinations with manual pipetting). Dilution of standards can be done in 1X PBS+ 0.1% Tween 20**.**

1) Preparation of 1X PBS+ 0.1% Tween 20:
Add 0.5 mL of 10X PBS to 4.5 mL H_2O then add 50µL of Tween 20 10%.

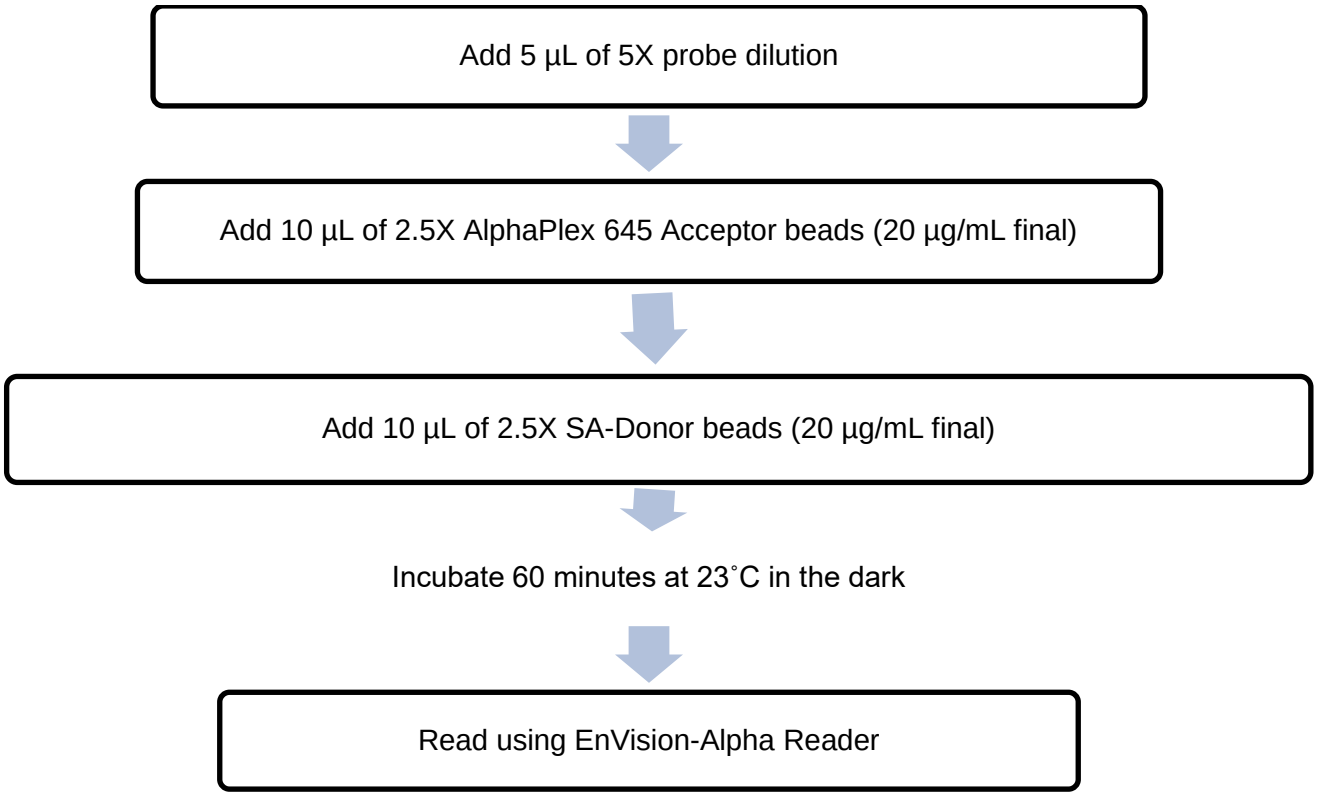
2) Preparation of 5x probe (biotinylated mIgG) dilutions:
Dilute probe to a 500 nM stock solution.
Prepare dilution series in 1X PBS + 0.1% Tween 20 as follows, changing tip for each dilution:

Tube	Volume of Probe	Volume of 1X Buffer (µL)	[Biotinylated mIgG] (M)	
			(in 5 µL 5X)	(25 µL Final Assay Volume)
A	4 µL of 12.5 µM	96	5E-07	1E-07
B	30 µL of tube A	70	1.5E-07	3E-08
C	30 µL of tube B	60	5E-08	1E-08
D	30 µL of tube C	70	1.5E-08	3E-09
E	30 µL of tube D	60	5E-09	1E-09
F	30 µL of tube E	70	1.5E-09	3E-10
G	30 µL of tube F	60	5E-10	1E-10
H	30 µL of tube G	70	1.5E-10	3E-11
I	30 µL of tube H	60	5E-11	1E-11
J	30 µL of tube I	70	1.5E-11	3E-12
K	30 µL of tube J	60	5E-12	1E-12
L	0	70	0	0

3) Preparation of 2.5X AlphaPlex 645 STREPTAVIDIN Acceptor beads (50 µg /mL)
Add 15 µL of 5 mg/mL AlphaPlex 645 STREPTAVIDIN acceptor beads to 1485 µL of PBS + 0.1% Tween 20

4) Preparation of 2.5X Alpha Donor Beads (50 µg/mL):
Keep the beads under subdued laboratory lighting.
Add 5 µL 5 mg/mL Alpha Donor beads to 495 µL of 1X PBS + 0.1% Tween 20

5) In a white opaque OptiPlate 384-well microplate:



Recommendations

- AlphaPlex 645 signal is detected using an EnVision Multilabel Reader equipped with the Alpha option using the following settings: Total Measurement Time: 1000 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D670as (Barcode# 605), Emission Filter: Wavelength 570nm, bandwidth: 100nm, Transmittance 75%, (Barcode# 224).
- Alpha signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.
- Sodium azide should not be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal.
- **Important: All assays involving AlphaPlex 645 beads must be read with a D670 mirror instead of the regular D640 mirror used for regular Alpha.**

Suggested Materials and Instrumentation

Please visit our website www.perkinelmer.com/AlphaTech

You will find detailed recommendations for common situations you might encounter with your Alpha Assay kit at:

http://www.perkinelmer.com/in/resources/technicalresources/applicationsupportknowledgebase/alphalisa-alphascreen-no-washassays/alpha_troubleshoot.xhtml

RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.