TECHNICAL DATA SHEET

AlphaPlex™ Research Reagents

AlphaPlex 645 anti-FLAG Acceptor Beads

Product number: AP112Sm-C/M/R

Lot number: 2695009

Manufacturing date: March 4, 2020

Research Use Only. Not for use in diagnostic procedures.

Product Information

Description: AlphaPlex-645 anti-FLAG Acceptor Beads at 5 mg/mL in PBS pH 7.2 supplemented with 0.05% Kathon as a preservative.

Application: This product is designed for use as a tool to generate Alpha assays involving peptides and proteins fused with the FLAG epitope tag (sequence (M)DYKDDDDK). This tag is quite popular as it is small, unknown in nature and is coupled with highly specific antibodies

Formats:

Catalog #	Size	Volume	Assay Points
AP112Sm-C	250 µg	50 µL	500
AP112Sm-M	5 mg	1000 µL	10 000
AP112Sm-R	25 mg	5000 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 6 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Sensitivity: EC_{50}: 2 nM peptide

Minimal signal: 5 000 counts*

Maximal signal: 380 000 counts*

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

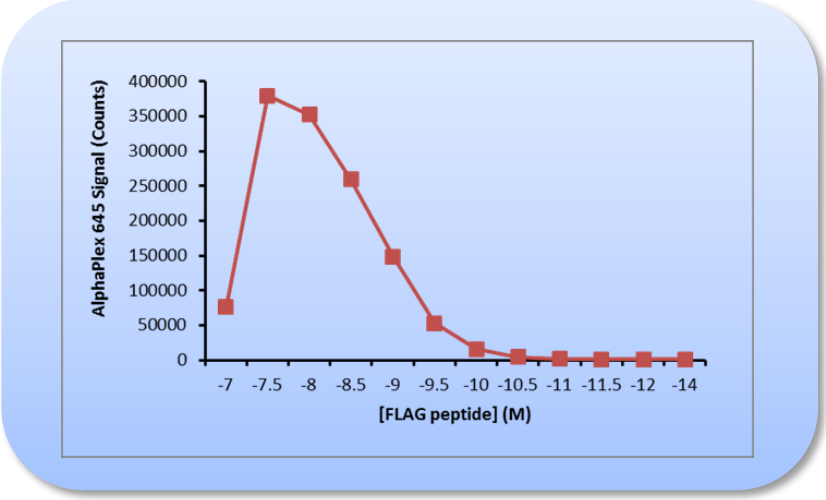


Figure. 1. Typical assay curve. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader with Alpha option 2104. The curve was obtained by mixing anti-FLAG acceptor and streptavidin donor beads with increasing concentrations of biotinylated FLAG peptide. 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}: 0.92 nM

Minimal signal: 120 counts

Maximal signal: 84581 counts

Protocol for ANTI-FLAG Toolbox Acceptor Beads Quality Control Assay
ANTI-FLAG protocol (ANTI-FLAG incubation steps) – Dilution of standards in PBS + 0.1% Tween 20 (buffer).

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.

Steps for Preparing Reagents

If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.

1) Preparation of PBS + 0.1% Tween 20:
   Add 0.1mL of 10%Tween 20 to 10mL PBS.

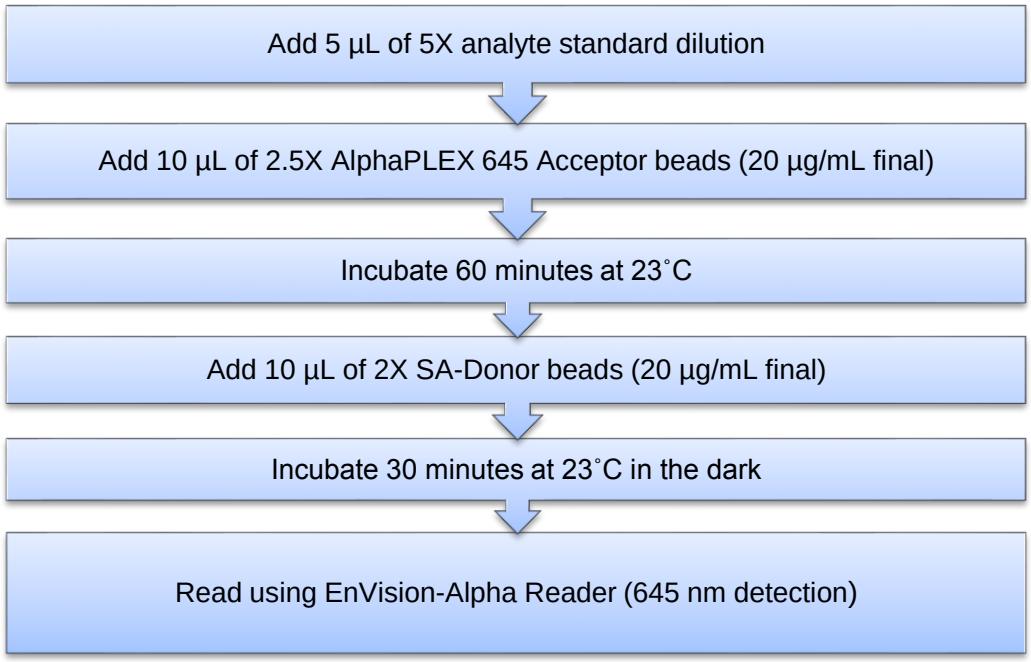
2) Preparation of 5x analyte (biotinylated ANTI-FLAG) dilutions:
   a. Dilute probe to a 500 nM stock solution: Take 5µL of 100µM stock into 95µL of PBS + 0.1% Tween 20
   b. Prepare dilution series in PBS + 0.1% Tween 20 as follows, changing tip for each dilution:

Tube	Volume of Analyte	Volume of Buffer (µL)	[Biotinylated ANTI-FLAG] (M)	
			(in 5 µL 5X)	(25 µL Final Assay Volume)
A	10 µL of 5 µM	90	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	100	0	0

3) Preparation of 2.5X AlphaPlex 645 Anti-ANTI-FLAG Acceptor beads (50 µg /mL)
Add 15 µL of 5 mg/mL AlphaPlex 645 Anti-ANTI-FLAG 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 PBS + 0.1% Tween 20

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



Important: 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# 244).

Recommendations

- 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 Alpha signal.

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

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