

AlphaPlex™-545

Anti-FLAG Acceptor Beads

Product number: AP112TB-C **Lot Number:** 3229306

Material provided: AlphaPlex-545 anti-FLAG Acceptor Beads at 5 mg/mL in PBS pH 7.2 supplemented with 0.05% Kathon as a preservative.

Product Format: AP112TB-C: 250 µg, 50 µL, 500 assay points

AP112TB-M: 5 mg, 1 mL, 10 000 assay points

AP112TB-R: 25 mg, 5 mL, 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: August 22, 2023 **Document version:** 1

Product Information

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

Storage: Store product 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.

Quality Control

Lot to lot consistency is confirmed in an Alpha assay. Maximum and minimum signals and EC50 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

EC₅₀: 1.03 nM
Min counts: 300 counts
Max counts: 331,206 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).

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 5 μ L of 100 μ M stock into 95 μ L 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 (μ L)	[Biotinylated ANTI-FLAG] (M)	
			(in 5 μ L 5X)	(25 μ L Final Assay Volume)
A	10 μ L of 5 μ M	90	5E-07	1E-07
B	30 μ L of tube A	70	1.5E-07	3E-08
C	30 μ L of tube B	60	5E-08	1E-08
D	30 μ L of tube C	70	1.5E-08	3E-09
E	30 μ L of tube D	60	5E-09	1E-09
F	30 μ L of tube E	70	1.5E-09	3E-10
G	30 μ L of tube F	60	5E-10	1E-10
H	30 μ L of tube G	70	1.5E-10	3E-11
I	30 μ L of tube H	60	5E-11	1E-11
J	30 μ L of tube I	70	1.5E-11	3E-12
K	30 μ L of tube J	60	5E-12	1E-12
L	0	100	0	0

3) Preparation of 2.5X AlphaPlex 545 Anti-ANTI-FLAG Acceptor beads (50 μ g /mL)

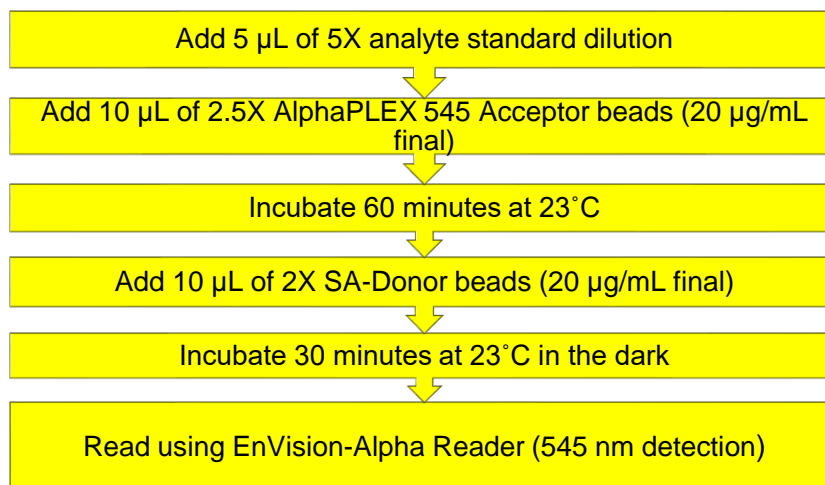
Add 15 μ L of 5 mg/mL AlphaPlex 545 Anti-ANTI-FLAG acceptor beads to 1485 μ L of PBS + 0.1% Tween 20

4) Preparation of 2.5X Alpha Donor Beads (50 μ g/mL):

Keep the beads under subdued laboratory lighting.

Add 5 μ L 5 mg/mL Alpha Donor beads to 495 μ L of PBS + 0.1% Tween 20

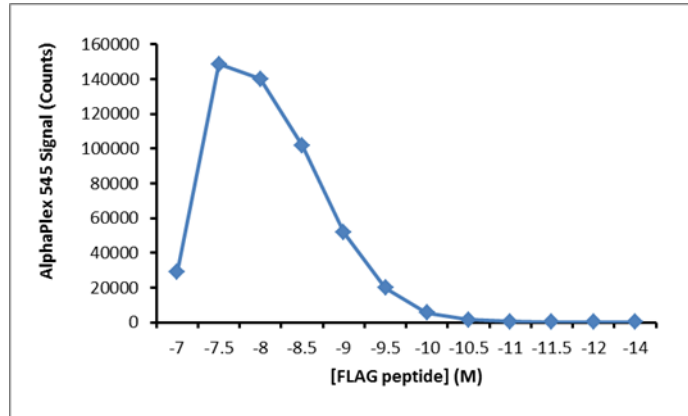
5) In a white opaque OptiPlate 384-well microplate:



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.

Typical Product Data



Typical assay curve. The data was generated using a white Optiplate™-384 microplate and the EnVision® Reader. The curve was obtained by mixing anti-FLAG acceptor and streptavidin donor beads with increasing concentrations of biotinylated FLAG peptide. The EC₅₀ was measured from the curve portion ranging from 0 analyte to the hook point.

Please visit our website for additional information on AlphaPlex technology at www.revvy.com

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AP112TB-R Rev01

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