

AlphaScreen®

FLAG Detection Kit

Product number: 6760613C

Lot Number: 3347486

Product Format: 6760613C: 500 assay points

6760613M: 10 000 assay points

6760613R: 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: November 8, 2024

Document version: 1

Kit Components

Component	6760613C	6760613M	6760613R
Anti-FLAG Acceptor Beads at 5 mg/mL in 1X PBS, 0.05% Kathon, pH 7.2	1 x 50 µL (6760128)	1 x 1 mL (6760129)	1 x 5 mL (6760129B)
Streptavidin Donor Beads at 5 mg/mL in 1X PBS, 0.05% Kathon, pH 7.2	1 x 50 µL (6760003)	1 x 1 mL (6760004)	1 x 5 mL (6760004B)
Biotinylated-FLAG at 5 µM in 1X PBS, 0.05% Kathon, pH 7.2	1 x 50 µL (6760276)	1 x 50 µL (6760276)	1 x 50 µL (6760276)
10x Buffer : 10X PBS, 0.05% Kathon, pH 7.2	1 x 1.5 mL (6760021G)	1 x 1.5 mL (6760021G)	1 x 1.5 mL (6760021G)

Product Information

Antibody/Protein: The FLAG antibody is a mouse monoclonal antibody (IgG₁) that recognizes the FLAG sequence at the N-terminus, Met-N-terminus, C-terminus, or at an internal site of FLAG fusion protein.

Stability: This kit is stable for at least 15 months from the date of manufacture when stored in its original packaging and the recommended storage conditions.

Storage: Store undiluted at 4°C protected from light. Freeze-thaw is not recommended and cause the beads to form aggregates.

Recommended Use: AlphaScreen® 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.

Quality Control

Alpha maximum signal, minimum signal and EC50 are determined using a biotinylated- FLAG titration assay performed on an EnVision® instrument. We certify that these results meet our requirements.

Maximum Signal:	957781 counts
Minimum Signal:	963 counts
EC ₅₀ :	5.30 nM

Recommended Assay Conditions

Note: This protocol provides a method to verify kit performance and is not representative of an assay. Sufficient biotinylated-probe and 10x buffer is provided to perform 3 titration curves in triplicate as described.

1x Buffer:	Add 500 µL 10x buffer to 4.5 mL Milli-Q® H ₂ O (or equivalent). Add 5 mg BSA (0.1% final concentration) and adjust pH to 7.4.
Acceptor Beads:	Add 5 µL Anti-FLAG Acceptor beads to 245 µL 1x buffer.
Donor beads:	Add 5 µL Streptavidin Donor beads to 245 µL 1x buffer.
Biotinylated-probe:	From the 5 µM biotin-FLAG, prepare a ½ log dilution series (1.5 µM to 0.15 NM) in 1x buffer. Include a buffer only control.

Titration Protocol

To a white opaque OptiPlate-384:

- 1) Add 5 µL biotin-FLAG dilutions (from lowest to highest concentration).
- 2) Add 10 µL of 1x buffer
- 3) Pre-mix (1:1) the Anti-FLAG Acceptor beads and Streptavidin Donor beads from previously diluted solutions by adding 200 µL Anti-FLAG Acceptor beads to 200 µL Streptavidin Donor beads.
- 4) Add 10 µL of pre-mix (1:1) of Anti-FLAG Acceptor beads and Streptavidin Donor beads.

Incubate in the dark at room temperature for 60 minutes and analyze on your Alpha capable detection reader.

Typical Product Data

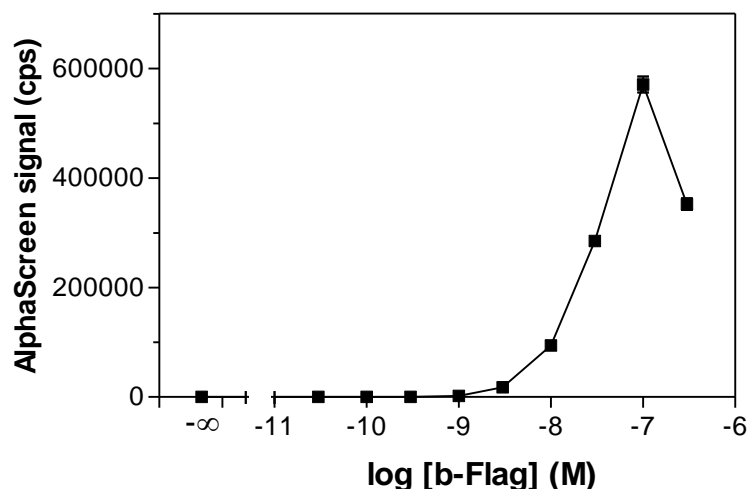


Figure 1: Biotinylated-probe titration assay 384-well biotinylated-FLAG titration curve (25 μ L final volume; Reader: Envision). Note: Alpha signal will vary depending on instrument detection protocol, incubation temperature and incubation time.

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

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