

Caution: For Laboratory Use. A research reagent for research purposes only

## FLAG Detection Kit

**Product No.:** 6760613C/M/R

**Lot No.:** 676-547-A

### Material Provided

**Format:**

6760613C	500 Assay Points
6760613M	10000 Assay Points
6760613R	50000 Assay Points

Note: The number of assay points is based on a final bead concentration of 20 µg/mL in a 25 µL/well reaction volume.

**Manufacturing Date:** June 22, 2011

#### Kit Components:

Component	6760613C	6760613M	6760613R
<b>Anti-FLAG Acceptor Beads at 5 mg/mL in 1X PBS, 0.05% Proclin-300, pH 7.2</b>	1 x 50 µL (6760128)	1 x 1 mL (6760129)	1 x 5 mL (6760129B)
<b>Streptavidin Donor Beads at 5 mg/mL in 1X PBS, 0.05% Proclin-300, pH 7.2</b>	1 x 50 µL (6760003)	1 x 1 mL (6760004)	1 x 5 mL (6760004B)
<b>Biotinylated-FLAG at 1.5 µM in 1X PBS, 0.05% Proclin-300, pH 7.2</b>	1 x 50 µL (6760276)	1 x 50 µL (6760276)	1 x 50 µL (6760276)
<b>10x Buffer : 10X PBS, 0.05% Proclin-300, pH 7.2</b>	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 (IgG<sub>1</sub>) 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 product is stable for at least **15 months** from the manufacturing date if used and stored under recommended conditions.

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

**Recommended use:** AlphaScreen® donor beads are **light sensitive** and should be handled under subdued or green filtered light conditions (< 100 Lux). Vortex beads prior to use.

For additional information on running AlphaScreen® assays or on potential interfering compounds, please visit our website: [www.perkinelmer.com/AlphaTech](http://www.perkinelmer.com/AlphaTech)

## Quality Control

AlphaScreen<sup>®</sup> maximum signal, minimum signal and EC<sub>50</sub> are determined using a biotinylated-FLAG titration assay performed on an EnVision<sup>®</sup> HTS Alpha detection instrument. We certify that these results meet our requirements.

<u>TEST</u>	<u>RESULTS</u>
Maximum signal	250 800 cps
Minimum signal	472 cps
EC <sub>50</sub>	8.61 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<sup>®</sup> H<sub>2</sub>O (or equivalent). Add 5 mg BSA (0.1% final concentration) and adjust pH to 7.2.
- 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 1.5 µM biotin-FLAG, prepare a ½ log dilution series (0.15 µM to 15 pM) in 1x buffer. Include a buffer only control.

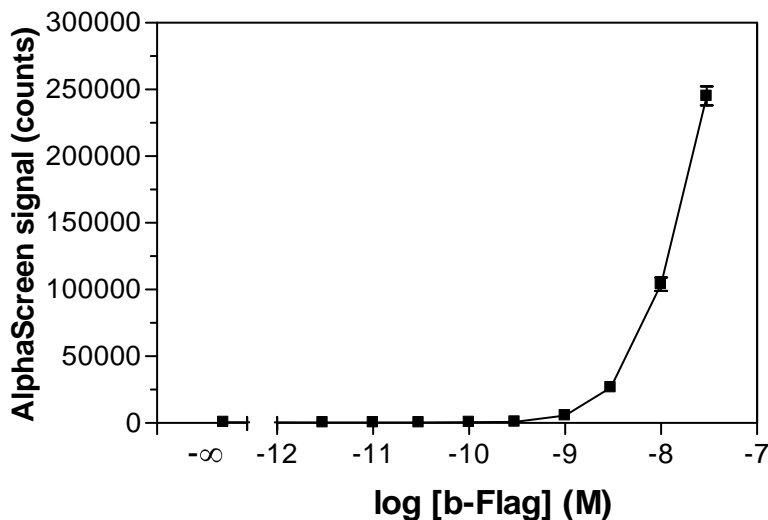
### Titration Protocol:

To a white opaque 384-well Optiplate:

- 1- Add 5 µL biotinylated-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 AlphaScreen<sup>®</sup> detection reader.

## Product Typical Data



**Figure 1: Biotinylated-probe titration assay**  
384-well biotinylated-FLAG titration curve (25  $\mu$ L final volume; Reader: Envision HTS Alpha).

**Note:** AlphaScreen<sup>®</sup> signal will vary depending on instrument detection protocol, incubation temperature and incubation time.

## Suggested Materials and Instrumentation

Please visit our website

[www.perkinelmer.com/AlphaTech](http://www.perkinelmer.com/AlphaTech)

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