

AlphaScreen®

## FITC (Fluorescein) Detection Kit

**Product number:** 6760605C **Lot Number:** 3308567

**Product Format:** 6760605C: 500 assay points  
 6760605M: 10 000 assay points  
 6760606R: 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:** 7/10/24 **Document version:** 1

### Kit Components

| Component   | 6760605C                 | 6760605M                 | 6760605R                 |
|---|--------------------------|--------------------------|--------------------------|
| Anti-FITC (Fluorescein) Acceptor Beads at 5 mg/mL in 25 mM Hepes, 100 mM              | 1 x 50 µL<br>(6760110)   | 1 x 1 mL<br>(6760111)    | 1 x 5 mL<br>(6760111B)   |
| Streptavidin Donor Beads at 5 mg/mL in 25 mM Hepes, 100 mM NaCl, 0.05% Kathon, pH 7.4 | 1 x 50 µL<br>(6760001)   | 1 x 1 mL<br>(6760002)    | 1 x 5 mL<br>(6760002B)   |
| Biotinylated-ERE-FITC at 0.05 µM in 25mM Hepes, 0.05% Kathon, pH 7.4                  | 1 x 50 µL<br>(6760260)   | 1 x 50 µL<br>(6760260)   | 1 x 50 µL<br>(6760260)   |
| 10x Buffer : 250 mM Hepes, 1 M NaCl, 0.05% Kathon, pH 7.4                             | 1 x 1.5 mL<br>(6760020G) | 1 x 1.5 mL<br>(6760020G) | 1 x 1.5 mL<br>(6760020G) |

### Product Information

**Antibody/Protein:** The FITC antibody is a mouse monoclonal antibody (IgG<sub>2a</sub>) that reacts with both free and conjugated FITC.

**Stability:** This kit is stable for at least 12 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 ERE-FITC titration assay performed on an EnVision® instrument. We certify that these results meet our requirements.

Maximum Signal: 911,550 counts  
Minimum Signal: 778 counts  
EC<sub>50</sub>: 0.12 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<sub>2</sub>O (or equivalent). Add 5 mg BSA (0.1% final concentration) and adjust pH to 7.4.

**Acceptor Beads:** Add 5 µL Anti-FITC (Fluorescein) Acceptor beads to 245 µL 1x buffer.

**Donor beads:** Add 5 µL Streptavidin Donor beads to 245 µL 1x buffer.

**Biotinylated-probe:** From the 0.05 µM biotin-ERE-FITC, prepare a ½ log dilution series (5 µM to 0.5 pM) in 1x buffer. Include a buffer only control.

## Titration Protocol

To a white opaque OptiPlate-384:

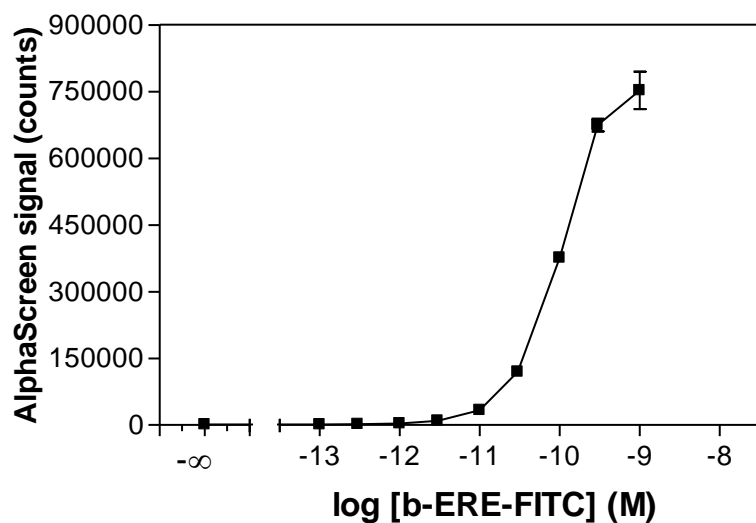
- 1) Add 5 µL biotin-ERE-FITC dilutions (from lowest to highest concentration).
- 2) Add 10 µL of 1x buffer
- 3) Add 5 µL of Anti-FITC IgG Acceptor beads.

Incubate in the dark at room temperature for 30 minutes

- 4) Add 5 µL of 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-ERE-FITC titration curve (25  $\mu$ L final volume; Reader: Envision). Note: Alpha signal will vary depending on instrument detection protocol, incubation temperature and incubation time.

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