cAMP AlphaScreen Assay Kit
Product No.: 6760635D/M/R
Lot No.: 2535111

Material Provided

Format:
- 6760635D: 1000 Assay Points
- 6760635M: 10000 Assay Points
- 6760635R: 50000 Assay Points

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

Manufacturing Date: February 25, 2019

Kit Components:

<table>
<thead>
<tr>
<th>Component</th>
<th>6760635D</th>
<th>6760635M</th>
<th>6760635R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-cAMP Acceptor Beads at 5 mg/mL in 1X PBS, 0.05% Proclin-300, pH 7.2</td>
<td>1 x 100 µL (6760100B)</td>
<td>1 x 1 mL (6760101)</td>
<td>1 x 5 mL (6760101B)</td>
</tr>
<tr>
<td>Streptavidin Donor Beads at 5 mg/mL in 1X PBS, 0.05% Proclin-300, pH 7.2</td>
<td>1 x 100 µL (6760003B)</td>
<td>1 x 1 mL (6760004)</td>
<td>1 x 5 mL (6760004B)</td>
</tr>
<tr>
<td>Biotinylated-cAMP Tracer Solid</td>
<td>1 x 10 nmol (6760301)</td>
<td>1 x 10 nmol (6760301)</td>
<td>1 x 65 nmol (6760301B)</td>
</tr>
<tr>
<td>cAMP standard at 50 µM</td>
<td>1 x 1 mL (FP1283)</td>
<td>1 x 1 mL (FP1283)</td>
<td>1 x 1 mL (FP1283)</td>
</tr>
<tr>
<td>10x Buffer : AlphaLISA Immunoassay Buffer**</td>
<td>1 x 2 mL (6760040G)</td>
<td>1 x 10 mL (6760040G)</td>
<td>1 x 100 mL (6760040G)</td>
</tr>
</tbody>
</table>

** Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).
1X PBS ph 7.4 is not included. It can be ordered from Gibco (cat# 10010-023)

Product Information

Antibody/Protein: The cAMP antibody is a rabbit monoclonal antibody highly specific for cyclic AMP. Its cross-reactivity with cGMP is < 0.005%.

Stability: This product is stable for at least 12 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. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
For additional information on running AlphaScreen® assays or on potential interfering compounds, please visit our website: www.perkinelmer.com/AlphaTech

Quality Control

AlphaScreen® maximum signal, minimum signal and IC₅₀ are determined by a cAMP competition assay performed on an EnVision® HTS Alpha detection instrument. We certify that these results meet our requirements.

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum signal</td>
<td>68,306</td>
</tr>
<tr>
<td>Minimum signal</td>
<td>258</td>
</tr>
<tr>
<td>LDL (nM)</td>
<td>0.860</td>
</tr>
<tr>
<td>IC₅₀ (nM)</td>
<td>3.48</td>
</tr>
</tbody>
</table>

Recommended Assay Conditions

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

1x Buffer: Add 500 μL 10x buffer to 4.45 mL Milli-Q® H₂O (or equivalent).

Acceptor Beads: Add 5 μL Anti-cAMP Acceptor beads to 495 μL 1x buffer.

biotinylated cAMP-Tracer: Dissolve biotinylated cAMP-Tracer (10 nmol) in 1 mL 1X PBS (final 10 μM) Further dilute 10X in 1 X PBS, pH 7.4 to obtain a 1 μM solution. *Solids may not be visible deposited along the walls of the tube*

Donor beads: Add 6.25 μL Streptavidin Donor beads to 579.75 μL 1x buffer.

cAMP standard: From the 50 μM cAMP standard, prepare a ½ log dilution series (5 μM to 50 pM) in 1x buffer. Include a buffer only control.

Titration Protocol:

To a white opaque 384-well Optiplate:

1- Add 10 μL of Anti-cAMP Acceptor beads.
2- Add 5 μL cAMP standard dilutions (from lowest to highest concentration).
3- Pre-mix the biotinylated cAMP-Tracer and Streptavidin Donor beads from previously diluted solutions by adding 39 μL of biotinylated cAMP-Tracer to the Streptavidin Donor beads dilution.

Incubate the premix and the plate in the dark at room temperature for 30 minutes.
4- Add 10 μL of premix biotinylated cAMP-Tracer /Streptavidin Donor beads.

Incubate in the dark at room temperature for 60 minutes and analyze on your AlphaScreen® detection reader.
Figure 1: Competition assay
384-well cAMP competition curve (25 μL final volume; Reader: Envision HTS Alpha).

Note: AlphaScreen® 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

This product is not for resale or distribution except by authorized distributors.

LIMITED WARRANTY: PerkinElmer BioSignal Inc. warrants that, at the time of shipment, the products sold by it are free from defects in material and workmanship and conform to specifications which accompany the product. PerkinElmer BioSignal Inc. makes no other warranty, express or implied with respect to the products, including any warranty of merchantability or fitness for any particular purpose. Notification of any breach of warranty must be made within 60 days of receipt unless otherwise provided in writing by PerkinElmer BioSignal Inc. No claim shall be honored if the customer fails to notify PerkinElmer BioSignal Inc. within the period specified. The sole and exclusive remedy of the customer for any liability of PerkinElmer BioSignal Inc. of any kind including liability based upon warranty (express or implied whether contained herein or elsewhere), strict liability contract or otherwise is limited to the replacement of the goods or the refunds of the invoice price of goods. PerkinElmer BioSignal Inc. shall not in any case be liable for special, incidental or consequential damages of any kind.