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CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaScreen®

cAMP Detection Kit

Product number: 6760635R **Lot Number:** 3350042

Product Format: 6760635D: 1000 assay points

6760635M: 10 000 assay points

6760635R: 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 22, 2024 **Document version:** 1

Kit Components

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

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

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, LDL and IC50 are determined by a cAMP competition assay performed on an EnVision® detection instrument. We certify that these results meet our requirements.

Maximum Signal::	190711 counts
Minimum Signal:	709 counts
LDL:	0.89 nM
IC50:	5.67 nM

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 into 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 OptiPlate-384:

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 pre-mix 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 Alpha capable detection reader.

Typical Product Data

Figure 1: Competition assay
384-well cAMP competition curve (25 µL 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.revvity.com

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