cAMP-Glo™ Assay

INSTRUCTIONS FOR USE OF PRODUCTS V1501, V1502 AND V1503.

Quick PROTOCOL

Preparing for the cAMP-Glo™ Assay

The cAMP-Glo™ Assay can be performed with adherent, suspension or frozen cells. For more information about cell preparation, see the *cAMP-Glo*™ Assay *Technical Bulletin* #TB357.

Reagent Preparation

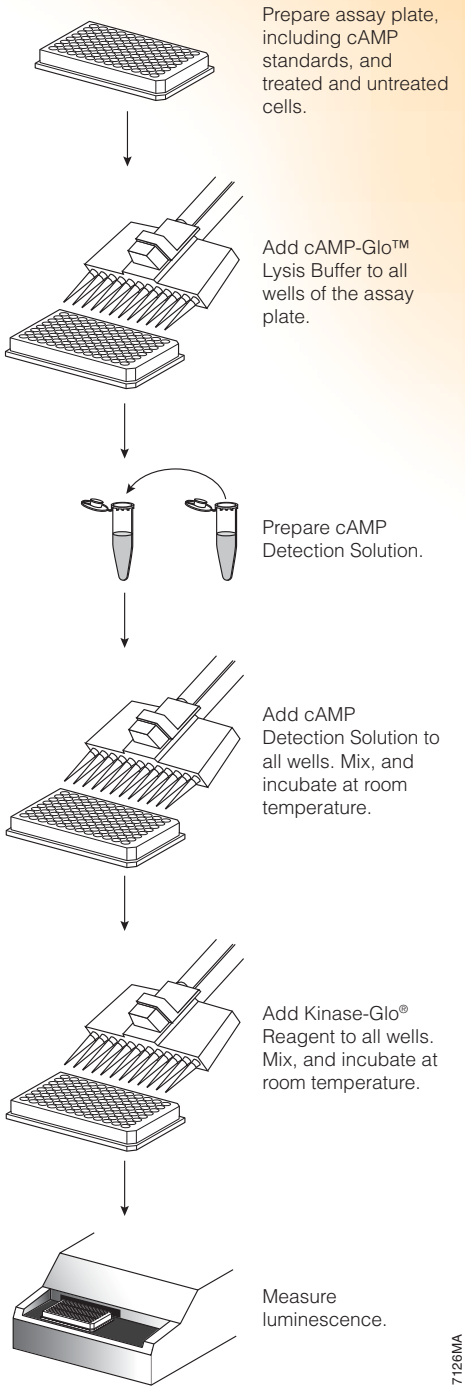
1. Prepare a 4.0µM cAMP solution by combining 250µl of induction buffer and 1.0µl of 1mM cAMP. Vortex to mix.
2. Transfer the entire volume of Kinase-Glo® Buffer into the amber bottle containing the Kinase-Glo® Substrate to form the Kinase-Glo® Reagent. Mix gently.

Generating a cAMP Standard Curve

3. Add 100µl of induction buffer to wells A2 through A12 of a 96-well plate.
4. Add 200µl of the 4.0µM cAMP solution to well A1.
5. Perform a serial twofold dilution of the 4.0µM cAMP solution in wells A1 through A11 of a 96-well plate. Do not add cAMP solution to the no-cAMP control reaction in well A12.
6. When using 96-well assay plates, transfer 20µl of each cAMP standard to wells reserved for the cAMP standard curve. For 384-well plates, transfer 7.5µl. For low-volume 384-well and 1536-well plates, transfer 1.0µl.

cAMP-Glo™ Assay Protocol

1. Treat cells with an agonist or test compound in induction buffer for the desired length of time.
2. Add 20µl (96-well plates), 7.5µl (384-well plates) or 1.0µl (low-volume 384-well and 1536-well plates) of cAMP-Glo™ Lysis Buffer to all wells. Incubate the plate with shaking at room temperature for 15 minutes.
3. Prepare the cAMP Detection Solution by combining 2.5µl (96-well plates), 3.5µl (384-well plates) or 5.0µl (low-volume 384-well and 1536-well plates) of Protein Kinase A per 1.0ml of cAMP-Glo™ Reaction Buffer. Mix by inversion.
4. Add 40µl (96-well plates), 15µl (384-well plates) or 2.0µl (low-volume 384-well and 1536-well plates) of cAMP-Glo™ Detection Solution to all wells. Mix the plate by shaking for 30–60 seconds. Incubate the plate at room temperature for 20 minutes.
5. Add 80µl (96-well plates), 30µl (384-well plates) or 4.0µl (low-volume 384-well and 1536-well plates) of room-temperature Kinase-Glo® Reagent to all reactions. Mix the plate by shaking for 30–60 seconds, and incubate at room temperature for 10 minutes.
6. Measure the luminescence with a plate-reading luminometer.



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Determining EC_{50} Values

Preparing the Test Compound

1. For suspension cells, prepare a 2X stock solution of the test compound in induction buffer. For adherent cells, prepare a 1X stock solution. Mix well.
2. Perform a serial twofold dilution of test compound in wells A1 through A11 of a 96-well plate. Do not add test compound to the no-test compound control reaction in well A12.

Assay Protocol

1. When using a 96-well assay plate with suspension cells, transfer 10µl of the various concentrations of test compound to the assay plate. For 384-well plates, transfer 3.75µl, and for low-volume 384-well and 1536-well plates, transfer 0.5µl. Add an equal volume of cell suspension.

   When using a 96-well assay plate with adherent cells, transfer 20µl of the various concentrations of test compound to the assay plate containing cells. For 384-well plates, transfer 7.5µl, and for low-volume 384-well and 1536-well plates, transfer 1.0µl.
2. Mix the plate by shaking for 30–60 seconds. Incubate at room temperature for 15 minutes.
3. Add 20µl (96-well plates), 7.5µl (384-well plates) or 1.0µl (low-volume 384-well and 1536-well plates) of cAMP-Glo™ Lysis Buffer to all wells. Incubate the plate with shaking at room temperature for 15 minutes.
4. Prepare the cAMP Detection Solution by combining 2.5µl (96-well plates), 3.5µl (384-well plates) or 5.0µl (low-volume 384-well and 1536-well plates) of Protein Kinase A per 1.0ml of cAMP-Glo™ Reaction Buffer. Mix by inversion.
5. Add 40µl (96-well plates), 15µl (384-well plates) or 2.0µl (low-volume 384-well and 1536-well plates) of cAMP Detection Solution to all wells. Mix by shaking for 30–60 seconds. Incubate the plate at room temperature for 20 minutes.
6. Add 80µl (96-well plates), 30µl (384-well plates) or 4.0µl (low-volume 384-well and 1536-well plates) of room-temperature Kinase-Glo® Reagent to all wells. Mix plate by shaking for 30–60 seconds, and incubate the plate at room temperature for 10 minutes.
7. Measure luminescence in each well using a plate-reading luminometer.

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Determining IC_{50} Values

Preparing the Test Compound

1. For adherent cells, prepare a 1X stock solution of the test compound in induction buffer with an appropriate concentration of a receptor-specific agonist (e.g., 100nM SKF38393). For suspension cells, prepare a 2X stock solution. Mix well.
2. Prepare a serial twofold dilution of test compound in well A1 through A11 of a 96-well plate. Do not add test compound to the no-antagonist control reaction in well A12.

Assay Protocol

1. When using a 96-well assay plate with suspension cells, transfer 10µl of the various concentrations of test compound to the assay plate. For 384-well plates, transfer 3.75µl, and for low-volume 384-well and 1536-well plates, transfer 0.5µl. Add an equal volume of cell suspension.

   When using a 96-well assay plate with adherent cells, transfer 20µl of the various concentrations of test compound to the assay plate containing cells. For 384-well plates, transfer 7.5µl, and for low-volume 384-well and 1536-well plates, transfer 1.0µl.
2. Mix the plate by shaking for 30–60 seconds. Incubate at room temperature for 20 minutes.
3. Add 20µl (96-well plates), 7.5µl (384-well plates) or 1.0µl (low-volume 384-well and 1536-well plates) of cAMP-Glo™ Lysis Buffer to all wells. Incubate the plate with shaking at room temperature for 15 minutes.
4. Prepare the cAMP Detection Solution by combining 2.5µl (96-well plates), 3.5µl (384-well plates) or 5.0µl (low-volume 384-well and 1536-well plates) of Protein Kinase A per 1.0ml of cAMP-Glo™ Reaction Buffer. Mix by inversion.
5. Add 40µl (96-well plates), 15µl (384-well plates) or 2.0µl (low-volume 384-well and 1536-well plates) of cAMP Detection Solution to all wells. Mix by shaking for 30–60 seconds. Incubate the plate at room temperature for 20 minutes.
6. Add 80µl (96-well plates), 30µl (384-well plates) or 4.0µl (low-volume 384-well and 1536-well plates) of room-temperature Kinase-Glo® Reagent to all wells. Mix plate by shaking for 30–60 seconds, and incubate the plate at room temperature for 10 minutes.
7. Measure luminescence in each well using a plate-reading luminometer.

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Determining Z´ Factor

Reagent Preparation

1. Prepare a 100nM cAMP solution by combining 1mM cAMP and induction buffer as follows. Vortex to mix.

Component	96-Well Assay Plate	384-Well Plate	Low-Volume 384-Well and 1536-Well Plates
Induction Buffer	1,000µl	1,600µl	500µl
1mM cAMP	0.4µl	0.64µl	0.2µl

Assay Protocol

1. Prepare the no-cAMP reactions by adding 20µl (96-well plates), 7.5µl (384-well plates) or 1.0µl (low-volume 384-well and 1536-well plates) of induction buffer to each well in half of the assay plate.
2. Prepare the cAMP reactions by adding 20µl (96-well plates), 7.5µl (384-well plates) or 1.0µl (low-volume 384-well and 1536-well plates) of 100nM cAMP to wells in the other half of the plate.
3. Add 20µl (96-well plates), 7.5µl (384-well plates) or 1.0µl (low-volume 384-well and 1536-well plates) of cAMP-Glo™ Lysis Buffer to all wells. Incubate the plate with shaking at room temperature for 15 minutes.
4. Prepare the cAMP Detection Solution by combining 2.5µl (96-well plates), 3.5µl (384-well plates) or 5.0µl (low-volume 384-well and 1536-well plates) of Protein Kinase A per 1.0ml of cAMP-Glo™ Reaction Buffer. Mix by inversion.
5. Add 40µl (96-well plates), 15µl (384-well plates) or 2.0µl (low-volume 384-well and 1536-well plates) of cAMP Detection Solution to all wells. Mix by shaking for 30–60 seconds. Incubate the plate at room temperature for 20 minutes.
6. Add 80µl (96-well plates), 30µl (384-well plates) or 4.0µl (low-volume 384-well and 1536-well plates) of room-temperature Kinase-Glo® Reagent to all wells. Mix plate by shaking for 30–60 seconds, and incubate the plate at room temperature for 10 minutes.
7. Measure luminescence in each well using a plate-reading luminometer.

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