cAMP-Glo™ Assay

Instructions for Use of Products V1501 and V1502.

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 each cAMP standard to wells reserved for the cAMP standard curve.
   96-well plate: Transfer 20µl of each cAMP standard.
   384-well plate: Transfer 7.5µl of each cAMP standard.
   Low-volume 384-well and 1,536-well plates: Transfer 1.0µl of each cAMP standard.

cAMP-Glo™ Assay Protocol

1. Treat cells with an agonist or test compound in induction buffer for the desired length of time.
2. Add cAMP-Glo™ Lysis Buffer to all wells. Incubate the plate with shaking at room temperature for 15 minutes.
   96-well plate: Add 20µl of cAMP-Glo™ Lysis Buffer.
   384-well plate: Add 7.5µl of cAMP-Glo™ Lysis Buffer.
   Low-volume 384-well and 1,536-well plates: Add 1.0µl of cAMP-Glo™ Lysis Buffer.
3. Prepare the cAMP Detection Solution by combining one of the following volumes of Protein Kinase A with 1.0ml of cAMP-Glo™ Reaction Buffer. Mix by inversion.
   96-well plate: Add 2.5µl of Protein Kinase A.
   384-well plate: Add 3.5µl of Protein Kinase A.
   Low-volume 384-well and 1,536-well plates: Add 5.0µl of Protein Kinase A.
4. Add 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.
   96-well plate: Add 40µl of cAMP Detection Solution.
   384-well plate: Add 15µl of cAMP Detection Solution.
   Low-volume 384-well and 1,536-well plates: Add 2.0µl of cAMP Detection Solution.
5. Add 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.
   96-well plate: Add 80µl of room-temperature Kinase-Glo® Reagent.
   384-well plate: Add 30µl of room-temperature Kinase-Glo® Reagent.
   Low-volume 384-well and 1,536-well plates: Add 4.0µl of room-temperature Kinase-Glo® Reagent.
6. Measure the luminescence with a plate-reading luminometer.

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. For suspension cells, transfer various concentrations of test compound to the assay plate. Add an equal volume of cell suspension.
   96-well plate: Transfer 10µl of test compound.
   384-well plate: Transfer 3.75µl of test compound.
   Low-volume 384-well and 1,536-well plates: Transfer 0.5µl of test compound.

   For adherent cells, transfer various concentrations of test compound to the assay plate containing cells.
   96-well plate: Transfer 20µl of test compound.
   384-well plate: Transfer 7.5µl of test compound.
   Low-volume 384-well and 1,536-well plates: Transfer 1.0µl of test compound.
2. Mix the plate by shaking for 30–60 seconds. Incubate at room temperature for 15 minutes.
3. Add cAMP-Glo™ Lysis Buffer to all wells. Incubate the plate with shaking at room temperature for 15 minutes.
   96-well plate: Add 20µl of cAMP-Glo™ Lysis Buffer.
   384-well plate: Add 7.5µl of cAMP-Glo™ Lysis Buffer.
   Low-volume 384-well and 1,536-well plates: Add 1.0µl of cAMP-Glo™ Lysis Buffer.
4. Prepare the cAMP Detection Solution by combining one of the following volumes of Protein Kinase A with 1.0ml of cAMP-Glo™ Reaction Buffer. Mix by inversion.
   96-well plate: Add 2.5µl of Protein Kinase A.
   384-well plate: Add 3.5µl of Protein Kinase A.
   Low-volume 384-well and 1,536-well plates: Add 5.0µl of Protein Kinase A.
5. Add cAMP Detection Solution to all wells. Mix by shaking for 30–60 seconds. Incubate the plate at room temperature for 20 minutes.
   96-well plate: Add 40µl of cAMP Detection Solution.
   384-well plate: Add 15µl of cAMP Detection Solution.
   Low-volume 384-well and 1,536-well plates: Add 2.0µl of cAMP Detection Solution.
6. Add 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.
   96-well plate: Add 80µl of room-temperature Kinase-Glo® Reagent.
   384-well plate: Add 30µl of room-temperature Kinase-Glo® Reagent.
   Low-volume 384-well and 1,536-well plates: Add 4.0µl of room-temperature Kinase-Glo® Reagent.
7. Measure luminescence in each well using a plate-reading luminometer.

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. 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. For suspension cells, transfer various concentrations of test compound to the assay plate. Add an equal volume of cell suspension.
   96-well plate: Transfer 10µl of test compound.
   384-well plate: Transfer 3.75µl of test compound.
   Low-volume 384-well and 1,536-well plates: Transfer 0.5µl of test compound.

   For adherent cells, transfer various concentrations of test compound to the assay plate containing cells.
   96-well plate: Transfer 20µl of test compound.
   384-well plate: Transfer 7.5µl of test compound.
   Low-volume 384-well and 1,536-well plates: Transfer 1.0µl of test compound.
2. Mix the plate by shaking for 30–60 seconds. Incubate at room temperature for 20 minutes.
3. Add cAMP-Glo™ Lysis Buffer to all wells. Incubate the plate with shaking at room temperature for 15 minutes.
   96-well plate: Add 20µl of cAMP-Glo™ Lysis Buffer.
   384-well plate: Add 7.5µl of cAMP-Glo™ Lysis Buffer.
   Low-volume 384-well and 1,536-well plates: Add 1.0µl of cAMP-Glo™ Lysis Buffer.
4. Prepare the cAMP Detection Solution by combining one of the following volumes of Protein Kinase A with 1.0ml of cAMP-Glo™ Reaction Buffer. Mix by inversion.
   96-well plate: Add 2.5µl of Protein Kinase A.
   384-well plate: Add 3.5µl of Protein Kinase A.
   Low-volume 384-well and 1,536-well plates: Add 5.0µl of Protein Kinase A.
5. Add cAMP Detection Solution to all wells. Mix by shaking for 30–60 seconds. Incubate the plate at room temperature for 20 minutes.
   96-well plate: Add 40µl of cAMP Detection Solution.
   384-well plate: Add 15µl of cAMP Detection Solution.
   Low-volume 384-well and 1,536-well plates: Add 2.0µl of cAMP Detection Solution.
6. Add 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.
   96-well plate: Add 80µl of room-temperature Kinase-Glo® Reagent.
   384-well plate: Add 30µl of room-temperature Kinase-Glo® Reagent.
   Low-volume 384-well and 1,536-well plates: Add 4.0µl of room-temperature Kinase-Glo® Reagent.
7. Measure luminescence in each well using a plate-reading luminometer.

cAMP-Glo™ Assay

Instructions for Use of Products V1501 and V1502.

Quick Protocol

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 1,536-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 induction buffer to each well in half of the assay plate.
   96-well plate: Add 20µl of induction buffer.
   384-well plate: Add 7.5µl of induction buffer.
   Low-volume 384-well and 1,536-well plates: Add 1.0µl of induction buffer.
2. Prepare the cAMP reactions by adding 100nM cAMP to wells in the other half of the plate.
   96-well plate: Add 20µl of 100nM cAMP.
   384-well plate: Add 7.5µl of 100nM cAMP.
   Low-volume 384-well and 1,536-well plates: Add 1.0µl of 100nM cAMP.
3. Add cAMP-Glo™ Lysis Buffer to all wells. Incubate the plate with shaking at room temperature for 15 minutes.
   96-well plate: Add 20µl of cAMP-Glo™ Lysis Buffer.
   384-well plate: Add 7.5µl of cAMP-Glo™ Lysis Buffer.
   Low-volume 384-well and 1,536-well plates: Add 1.0µl of cAMP-Glo™ Lysis Buffer.
4. Prepare the cAMP Detection Solution by combining one of the following volumes of Protein Kinase A with 1.0ml of cAMP-Glo™ Reaction Buffer. Mix by inversion.
   96-well plate: Add 2.5µl of Protein Kinase A.
   384-well plate: Add 3.5µl of Protein Kinase A.
   Low-volume 384-well and 1,536-well plates: Add 5.0µl of Protein Kinase A.
5. Add cAMP Detection Solution to all wells. Mix by shaking for 30–60 seconds. Incubate the plate at room temperature for 20 minutes.
   96-well plate: Add 40µl of cAMP Detection Solution.
   384-well plate: Add 15µl of cAMP Detection Solution.
   Low-volume 384-well and 1,536-well plates: Add 2.0µl of cAMP Detection Solution.
6. Add 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.
   96-well plate: Add 80µl of room-temperature Kinase-Glo® Reagent.
   384-well plate: Add 30µl of room-temperature Kinase-Glo® Reagent.
   Low-volume 384-well and 1,536-well plates: Add 4.0µl of room-temperature Kinase-Glo® Reagent.
7. Measure luminescence in each well using a plate-reading luminometer.

Additional protocol information in Technical Bulletin #TB357, available online at: **www.promega.com**

