Screen Quest™ Live Cell cAMP Assay Service Pack

Catalog number: 36382, 36383, 36384
Unit size: 100 Tests, 200 Tests, 1000 Tests

Component	Storage	Amount (Cat No. 36382)	Amount (Cat No. 36383)	Amount (Cat No. 36384)
Component A: Ga16 DNA	Freeze (< -15 °C), Minimize light exposure	1 vial (15 µg)	2 vials (15 µg/vial)	1 vial (150 µg)
Component B: Transfectamine™ 5000	Freeze (< -15 °C), Minimize light exposure	1 vial (75 µL)	1 vial (150 µL)	1 vial (750 µL)
Component C: Calbryte™ 520 NW	Freeze (< -15 °C), Minimize light exposure	1 vial	2 vials	1 vial
Component D: 10X Pluronic® F127 Plus	Freeze (< -15 °C), Minimize light exposure	1 bottle (1 mL)	2 bottles (1 mL/bottle)	2 bottle (10 mL)
Component E: HHBS	Freeze (< -15 °C), Minimize light exposure	1 bottle (9 mL)	2 bottles (9 mL/bottle)	1 bottle (100 mL)
Component F: DMSO	Freeze (< -15 °C)	1 vial (100 µL)	1 vial (100 µL)	1 vial (250 µL)

OVERVIEW

G protein coupled receptors (GPCR) are one of the largest receptor classes targeted by drug discovery programs. Calcium flux (coupled via Gq pathway) assay is a preferred method in drug discovery for screening GPCR targets. However, over 60% of the known GPCRs signal through adenylyl cyclase activity coupled to cAMP. Most of the existing cAMP assays not only require cell lysis but also lack both temporal and spatial resolution. Screen Quest™ Live Cell cAMP Assay Service Pack provides the real-time monitoring of intracellular cAMP change in a high-throughput format without a cell lysis step. The assay works through the cell lines that contain either an exogenous cyclic nucleotide-gated channel (CNGC) or the promiscuous G-protein, Gα16. The channel is activated by elevated levels of intracellular cAMP, resulting in ion flux and cell membrane depolarization which can be detected with either a fluorescent calcium (such as Calbryte 520 AM, Cal-520 AM, Fluo-8 AM, or Fluo-4 AM and corresponding no wash calcium kits) or a fluorescent membrane potential dye. Co-expression of Gα16 with specific non-Gq-coupled receptors will result in the generation of an intracellular calcium signal upon receptor stimulation. The Screen Quest™ Live Cell cAMP Assay Service Pack provides all the reagents needed for the measurement of intracellular cAMP changes with a FLIPR, a FDSS or other equivalent fluorescence microplate readers. It has been successfully used to measure Gs and Gi coupled GPCR activity.

AT A GLANCE

Protocol summary

1. Prepare cells for transfection
2. Prepare Transfectamine™ 5000-DNA mixture
3. Add Transfectamine™ 5000-DNA mixture to the cell culture, incubate overnight
4. Transfer the transfected cells to a 96-well plate 24-30 hours after transfection, and incubate the culture overnight
5. Add Calbryte™ 520 NW dye-loading solution
6. Incubate at room temperature or 37 °C for 30-60 minutes
7. Monitor the fluorescence intensity at Ex/Em = 490/525 nm

Important Note

Thaw the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	515 nm
Emission	525 nm
Excitation	490 nm
Recommended plate	Black wall/Clear bottom
Instrument specification(s)	Bottom read mode/Programmable liquid handling

Note: This kit is compatible with the following instruments: FDSS, FLIPR, ViewLux, NOVOStar, Array Scan, FlexStation, and IN Cell Analyzer.

CELL PREPARATION

1. Seed the cells such that they will be ~60-70% confluent at the time of transfection.
2. Replace with fresh growth medium before transfection. For example, replace with 2 mL of medium per well for 6-well plates and 6 mL of medium for 10 cm plates.

PREPARATION OF STOCK SOLUTIONS

Calbryte™ 520NW stock solution

1. Add 20 µL of DMSO (Component F) to the vial of Calbryte™ 520NW (Component C) for Cat# 36382 and 36383, or 200 µL for Cat# 36384, and mix thoroughly.

   Note: 20 µL of Calbryte™ 520NW stock solution is enough for one plate. Unused Calbryte™ 520NW stock solution can be aliquoted and stored at ≤ -20 °C for more than one month, provided the tubes are tightly sealed. Protect from light and avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

1X Assay Buffer

1. Combine 9 mL of HHBS (Component E) with 1 mL of 10X Pluronic® F127 Plus (Component D), and mix thoroughly.

Calbryte™ 520NW Working Solution

1. Add 20 µL of Calbryte™ 520 NW stock solution to 10 mL of 1X Assay Buffer, and mix well.

   Note: The working solution is stable for at least 2 hours at room temperature.

TRANSFECTAMINE™ 5000-DNA MIXTURE

1. Add 15 µL of ddH_2O to the vial of Ga16 DNA (Component A), mix well to have the final concentration of 1 µg/µL.

   Note: Skip this step for Cat# 36384. Component A is already provided in solution form and is ready to use.

2. Mix 3 µg of DNA [for example, 1.5 µg of Ga16 DNA (Component A) and 1.5 µg DNA of the GPCR that you are interested] with 200 µL of serum-free medium.

3. Add 9 µL of Transfectamine™ 5000 (Component B) to the mixture from Step 1.

4. Mix well and incubate at room temperature for 20 minutes.

   Note: The ratio of Transfectamine™ 5000 and DNA need to be optimized for different cell lines. In general, the ratio for Transfectamine™ 5000 Transfection Reagent (µL) to DNA (µg) should be 3-5 µL : 1 µg.

Table 1. Sample protocols for a 6-well plate and a 10 cm plate

Component	6 well plate (per well)	10 cm plate
Fresh culture medium	2 mL	6 mL
Plasmid	~3 µg	10~15 µg
Serum-free medium	200 µL	600 µL
Transfectamine™ 5000 Transfection Reagent	~9 µL	~30-45 µL

SAMPLE EXPERIMENTAL PROTOCOL

Transfection and Translocation protocol

1. Add Transfectamine™ 5000 -DNA mixture to the culture plate and incubate overnight.

   Note: The recombinant protein can start to be detected as early as 16 hours after transfection. The maximal expression level may be observed 72~96 hours after transfection.

2. Transfer the transfected cells to a 96-well plate 24-30 hours post transfection and incubate overnight.
   - **For adherent cells:** Plate cells overnight in the growth medium at 40,000 to 80,000 cells/well/100 µL for a 96-well plate.
   - **For non-adherent cells:** Centrifuge the cells from the culture medium and then suspend the cell pellet in cell growth medium or HHBS at 125,000 to 250,000 cells/well/100 µL for a 96-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.

   Note: Each cell line should be evaluated on the individual basis to determine the optimal cell density for the intracellular calcium mobilization.

Run calcium assay

1. Add 100 µL/well (96-well plate) of Calbryte™ 520NW working solution into the cell plate.

2. Incubate the dye-loading plate in a cell incubator for 30 minutes, and then incubate the plate at room temperature for another 15-30 minutes.

   Note: If the assay requires 37 °C, perform the experiment immediately without further room temperature incubation.

   Note: If the cells can function well at room temperature for longer time, incubate the cell plate at room temperature for 1 hour (It is recommended that the incubation time be no longer than 2 hours.)

3. Prepare the compound plate with HHBS or your desired buffer.

4. Run the calcium flux assay by monitoring the fluorescence intensity at Ex/Em = 490/525 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

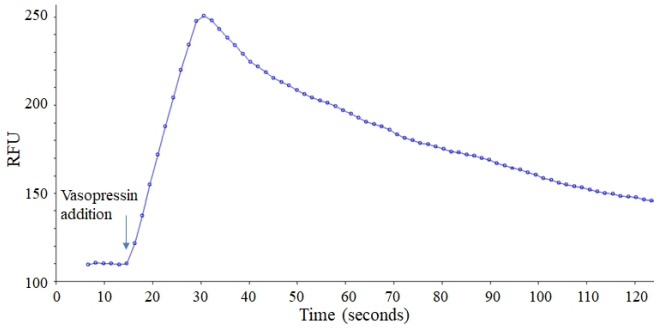

Figure 1. Vasopressin responses in CHO cells. CHO cells were transiently transfected with Ga16 and vasopressin receptor 2 (V2R). CHO cells were cultured in a 6-well plate and grown to ~60% confluence. Equal amounts of Ga16 (1.5 µg) and V2R plasmids (1.5 µg) were transfected with 9 µL of Transfectamine™ 5000. Cells were transferred to a 96-well plate at 50,000 cells/100 µL/well ~ 30 hours after transfection. 100 µL of Calbryte™ 520NW dye-loading solution was added ~ 48 hours after transfection and incubated at 37 °C for 45 minutes. Vasopressin (50 µL/well) was added using FlexStation 3 to achieve the final concentration of 100 nM.

DISCLAIMER

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