Non-Wash Fluorescent Calcium Dye Assay

CATALOG NUMBER: CA-C155-10, 10 plates; CA-C155-100, 100 plates

Description

Calcium ions (Ca^{2+}) is essential for living organisms, where movement of the calcium ion Ca^{2+} into and out of the cytoplasm functions as a signal for many cellular processes. Calcium is the fifth-most-abundant element by mass in the human body, where it is a common cellular ionic messenger with many functions, and serves also as a structural element in bone.

Elite™ Non-Wash Fluorescent Calcium Assay Kit provides a simple method for detecting calcium in physiology solutions by using our proprietary red fluorescence probe. The fluorescence signal can be easily read by a fluorescence microplate reader at Ex/Em = 485/530 nm. The kit allows homogeneous measurement of intracellular calcium changes caused by activation of G-protein coupled receptors or calcium channels. The assay involves only one step of dye addition and does not require any washing steps. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to high throughput automation.

Application

- High throughput screening of GPCR compounds using GPCR stable cell lines
- High throughput screening of calcium channel compounds

Features

- **Continuous**: Easily adapted to automation without a separation step.
- **Convenient**: Formulated to have minimal hands-on time. No interference with magnesium.
- **Non-radioactive**: No special requirement for waste disposal.

Kit Components

- **Component A:** Calcium Dye (light sensitive) — 10 vial (50 µg/vial)
- **Component B:** 10x Calcium Dye Signal Enhancer — 1 bottle (10 ml)

Storage

Component A: Keep in freezer (-20 °C) and avoid exposure to light and avoid of water.
Component B: Store at room Temperature.

Shelf Life

All reagents are stable for 6 months after receipt when stored properly at the recommended conditions

Materials Required (but not supplied)

- DMSO (Cat# D4540, Sigma) and Probenecid (Cat# P8761, Sigma).
- 96 or 384-well microplates: Tissue culture microplate with black wall and clear bottom is recommended.

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Assay Protocol for 96-Well/or 384-Well Plate

Thaw all the kit components to room temperature before starting the experiment.

1. **Prepare the cell culture plate:**
   1.1 Seed 80 µl of cell suspension into each well of a 96-well plate or 20 µl of cell suspension into each well of a 384-well plate.
   1.2 Grow the cells overnight in a CO_2 incubator
2. **Prepare assay buffers**:
   On the 2nd day:
   2.1 Prepare Buffer A (1X HBSS with 20 mM HEPES):
   10 ml of 1M HEPES, pH 7.3 + 490 ml of 1X HBSS.
   2.2 Prepare 1 ml of 500 mM Probenecid.
   Dissolve 142 mg of Probenecid in 1 ml of 1N NaOH.
   2.3 Prepare calcium dye stock solution.
   Add 8 µl of DMSO into the vial (**Component A**) containing 50 µg of calcium dye.
   2.4 Prepare **2X Dye Loading Buffer** (1 plate).
   Add 0.8 ml of 10X Calcium Dye Signal Enhancer (**Component B**) into 7.2 ml of Buffer A.
   Add 80 µl of 500 mM Probenecid.
   Add 8 µl of calcium dye stock solution. Mix well by vortexing.
3. **Assay procedure:**
   3.1 Take the cell plate out from the incubator.
   3.2 Add same volume of **2X Dye Loading Buffer** into each well, 80 µl to a 96-well plate or 20 µl to a 384-well plate.
   3.3 Incubate at 37 °C incubator for 1 hr.
   3.4 Take the cells out of the incubator and leave at room temp (in the dark) for 30 min.
   3.5 Put the plate into the instrument for assay
   For assays performed on a FlexStation (MDS), use the following wavelength parameters. Excitation: 485 nm; Emission: 530 nm; Cutoff 515 nm
   Note. Dispense speed and height for compound additions need to be optimized for each instrument.

   Molecular Device's FLIPR and Hamamatsu's FDSS can also be used for the assay.

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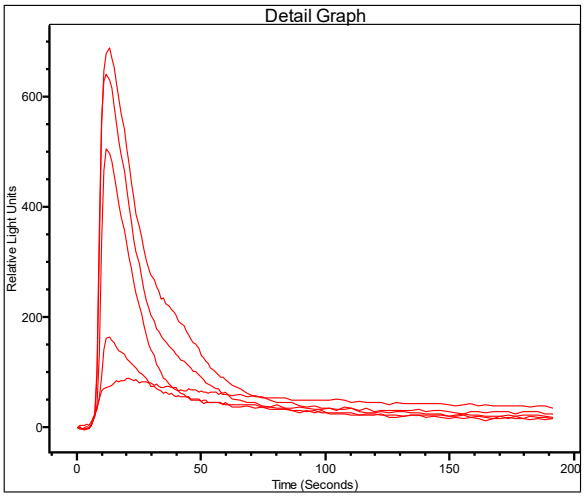

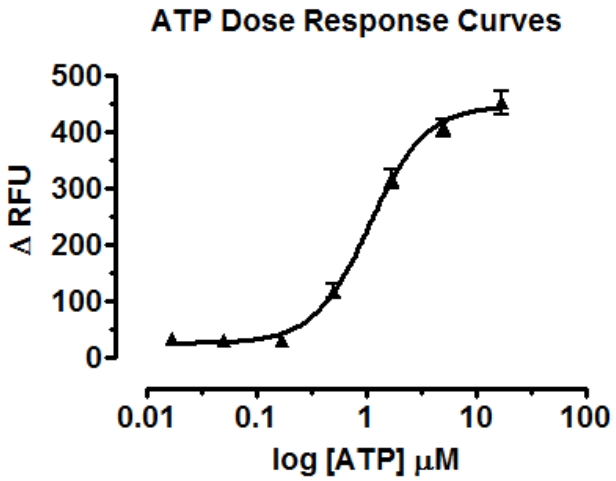

Figure 1. Response of endogenous P2Y receptors to ATP. HEK293 cells were plated overnight in 20 µl culture medium on a 384 well Biocoat poly-D lysine coated plate. The next day, the cells were dye-loaded by adding 20 µl of 2X Dye Loading Buffer and incubating for 1 hour at 37°C. ATP solution was added (10 µl/well) by a FLIPR Tetra (Molecular Devices), and the data was recorded simultaneously. **A**. Kinetic curve of calcium response to different concentrations of ATP. **B.** ATP dose response curve (n = 4). EC50 = 1.1 µM.

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