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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Elite™ Non-Wash Ratiometric Calcium Dye Assay (Fluorescence)

CATALOG NUMBER: CA-C166, 10 plates

Description

Calcium ions (Ca^{2+}) is essential for living organisms, where movement of the calcium ion Ca2+ 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.

Calcium plays an important role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and hormone secretion. The serum level of calcium is closely regulated within a limited range (9 to 10.5 mg/dL) in the human body. Both hypocalcemia and hypercalcemia are serious medical disorders. Causes of low calcium levels include chronic kidney failure, vitamin D deficiency, and low blood magnesium levels.

Elite™ Non-Wash Fluorescent Ratiometric Calcium Assay Kit allows homogeneous measurement of intracellular calcium changes caused by activation of G-protein coupled receptors or calcium channels. The ratio of 340/380 nm excitation allows accurate measurements of the intracellular Ca2+ concentration. Measuring by ratio considerably reduces the effects of uneven dye loading, leakage of dye, and photobleaching, as well as problems associated with measuring Ca2+ in cells of unequal thickness. The assay involves only one step of dye addition and does not require any washing steps. It is user friendly and cost effective. The assay can be easily implemented in a high throughput environment.

Application

- High throughput screening of GPCR compounds using ACTOne™ GPCR stable cell lines
- High throughput screening of GPCR compounds using AbPlus™ GPCR (Gi) stable cell lines

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.
- **Accurate**: Reduces the effects of uneven dye loading, leakage of dye, and photobleaching.

Kit Components and Storage

- **Component A:** Elite™ Calcium Dye (light sensitive) — 10 vials (50 ug/vial), to store at -20°C
- **Component B:** 10x Calcium Dye Signal Enhancer — 1 bottle (10 ml), to store at room temp.

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)
- 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 (optional).
   Dissolve 142 mg of Probenecid in 1 ml of 1N NaOH.
   2.3 Prepare calcium dye stock solution.
   Add 10 µl of DMSO into each vial containing the calcium dye for ONE plate.
   2.4 Prepare **2X Dye Loading Buffer** (for 1 plate).
   Add 1 ml of 10X Calcium Dye Signal Enhancer (**Component B**) into 9 ml of Buffer A.
   Add 100 µl of 500 mM Probenecid (optional).
   Add 10 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 (optional).
   3.5 Put the plate into the instrument for assay
   To perform the assays, use the following wavelength parameters:
   Excitation: 340 nm; Emission: 510 nm and
   Excitation: 380 nm; Emission: 510 nm
   Note. Dispense speed and height for compound additions need to be optimized for each instrument.

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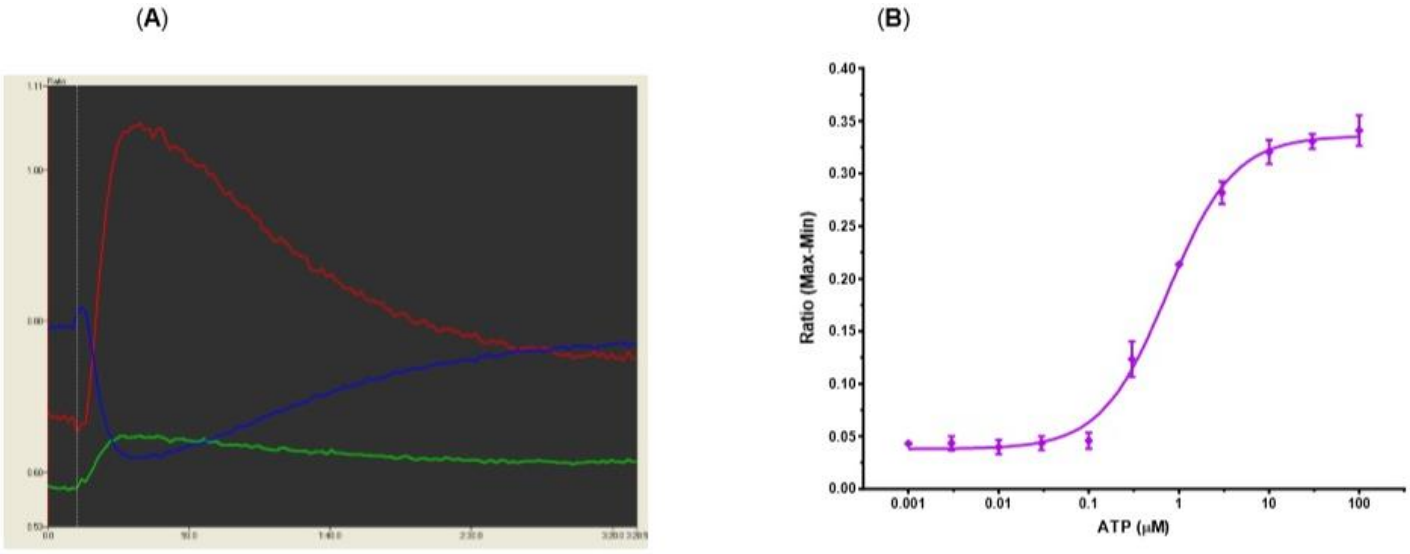

Figure 1. Response of endogenous P2Y receptors to ATP. HEK293 cells were plated overnight in 20 µl culture medium on a 384 well black/clear plate. The next day, the cells were dye-loaded by adding 20 µl of 2X Dye Loading Buffer and incubating for 1 hour at 37°C. ATP solution was added (10 µl/well) by a FDSS 7000 (Hamamatsu), and the data was recorded simultaneously. A. Kinetic curve of calcium response to 100 µM ATP (Green: emission at 340 nM; Blue: emission at 380 nM; Red: 340/380 ratio). B. ATP dose response curve (n = 4). EC50 = 0.75 µM