Technical Data Sheet

Codex Non-Wash Ratiometric Calcium Dye Kit

Product Information

Catalog Number: CB-80500-421
Size: Reagents for 10 plates
Components: Ratiometric Calcium Dye~50 μg, 10 vials, lyophilized (Part No: 80500-121)
10X Calcium Dye Signal Enhancer, 10 ml (Part No: 80500-112)

Description

The Codex Non-Wash RatiometricCalcium Dye 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.

Storage

Ratiometric Calcium Dye~50 μg	-20°C (protected from light)
10X Calcium Dye Signal Enhancer	Room Temp.

Materials not included

DMSO	Sigma D4540

ASSAY PROTOCOL

Prepare the cell plate:

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.
2. Grow the cells overnight in a CO2 incubator

Prepare the buffers (for one plate):

On the 2nd day:

1. Prepare Buffer A (1X HBSS with 20 mM HEPES):

   10 ml of 1M HEPES, pH 7.3 + 490 ml of 1X HBSS

2. Prepare 1 ml of 500 mM Probenecid (optional).

   Dissolve 142 mg of Probenecid in 1 ml of 1N NaOH

3. Prepare stock solution of calcium dye

   Add 10 μl of DMSO into each vial containing calcium dye for 1 plate

4. Prepare **2X Dye Loading Buffer** (1 plates).

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Add 1 ml of Codex 10X Calcium Dye Signal Enhancer 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.

Assay:

1. Take the cell plate out from the incubator.

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. Incubate at 37 °C incubator for 1 hr.

4. Take the cells out of the incubator and leave at room temp (in the dark) for 30 min (optional).

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

Appendix

(**A**) (**B**)

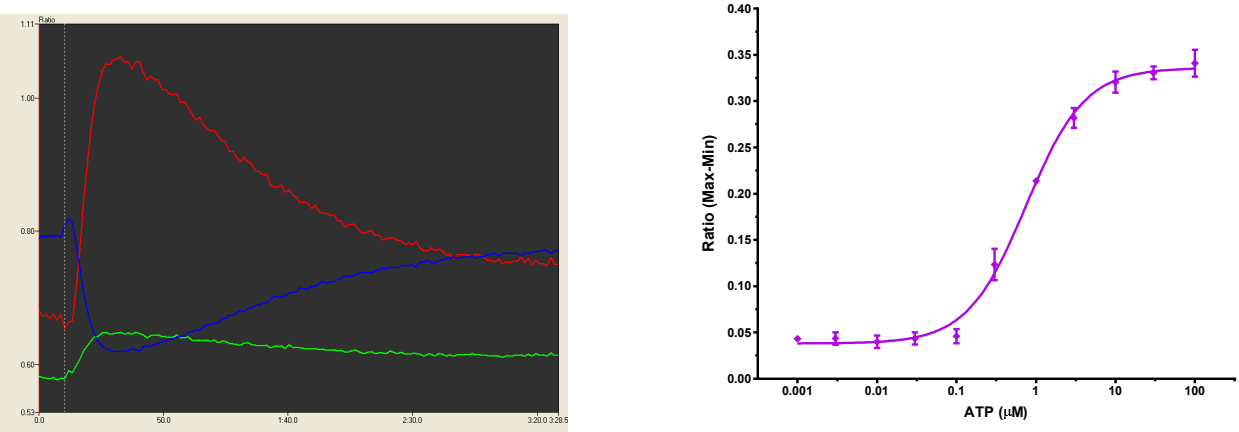

Figure 1. Response of endogenous P2Y receptors to ATP. HEK293 cells were plated overnight in 20 µl culture medium on a 384 well black/clear plate. The next day, the cells were dye-loaded by adding 20 µl of 2X Dye Loading Buffer and incubating for 1 hour at 37°C. ATP solution was added (10 µl/well) by 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.

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