

Technical Data Sheet

Codex ACTOne™ Non-Wash Calcium Dye Kit

Product Information

Catalog Number: CB-80500-301
Size: Reagents for 10 plates
Components: Codex Calcium Dye, 10 vials, lyophilized (Part No: 80500-110)
10X Calcium Dye Signal Enhancer, 10 ml (Part No: 80500-112)

Description

The Codex ACTOne™ Non-Wash Calcium Dye 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 is user friendly and cost effective. The assay can be easily implemented in a high throughput environment.

Storage

Codex Calcium Dye	-20°C (protected from light)
10X Calcium Dye Signal Enhancer	Room Temp.

Materials not included

DMSO	Sigma D4540
Probenecid	Sigma P8761

ASSAY PROTOCOL

Prepare the cell plate:

1. Seed 80 μ l of cell suspension into each well of a 96-well plate or 20 μ l of cell suspension into each well of a 384-well plate.
2. Grow the cells overnight in a CO₂ incubator

Prepare the buffers:

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.
Dissolve 142 mg of Probenecid in 1 ml of 1N NaOH
3. Prepare stock solution of calcium dye
Add 8 μ l of DMSO into each well containing 50 μ g of calcium dye
4. Prepare **2X Dye Loading Buffer** (1 plates).
Add 0.8 ml of Codex 10X Calcium Dye Signal Enhancer into 7.2 ml of Buffer A.
Add 80 μ l of 500 mM Probenecid.
Add 8 μ l 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 μ l to a 96-well plate or 20 μ l 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.
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.*

Appendix

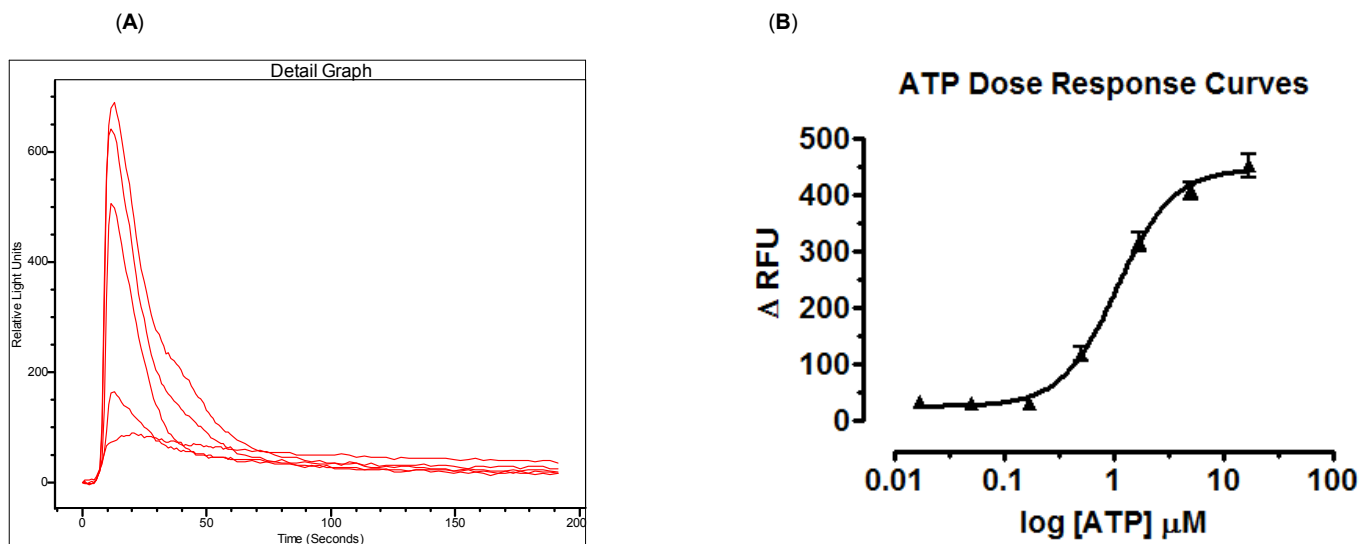


Figure 1. Response of endogenous P2Y receptors to ATP. HEK293 cells were plated overnight in 20 μ l culture medium on a 384 well Biocoat poly-D lysine coated plate. The next day, the cells were dye-loaded by adding 20 μ l of 2X Dye Loading Buffer and incubating for 1 hour at 37°C. ATP solution was added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

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