Technical Data Sheet

Codex ACTOne™ Non-Wash Calcium Dye Kit

Product Information

Catalog Number: CB-80500-305
Size: Reagents for 50 plates
Components: Codex Calcium Dye, 5 vials, lyophilized (Part No: 80500-113)
10X Calcium Dye Signal Enhancer, 50 ml (Part No: 80500-114)

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

Codex Biosolutions, **Inc**. 401 Professional Drive, Suite 160, Gaithersburg, MD 20879
Tel: (240)-632-8810; Fax: (240)-632-8820 E-Mail: orders@codexbiosolutions.com
http://www.codexbiosolutions.com/

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:

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 80 μl of DMSO into each well containing 0.5 mg of calcium dye
4. Prepare **2X Dye Loading Buffer** (1 plates).

   Add 8 ml of Codex 10X Calcium Dye Signal Enhancer into 72 ml of Buffer A.

   Add 800 μl of 500 mM Probenecid.

   Add 80 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.
5. Put the plate into the instrument for assay

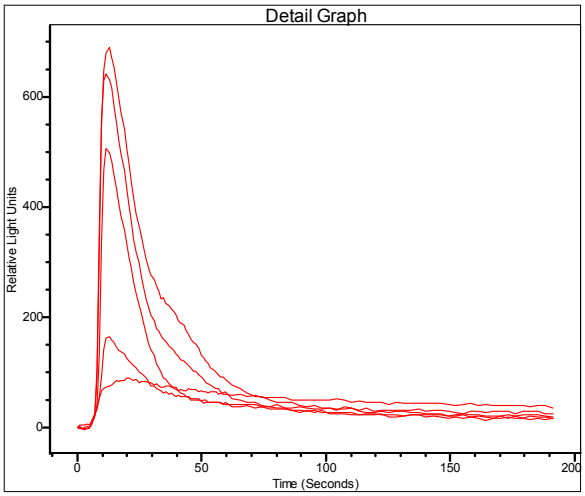
For assays performed on a FlexStation (MDS), use the following wavelength parameters. Excitation: 485 nm; Emission: 530 nm; Cutoff 515 nm

Note. Dispense speed and height for compound additions need to be optimized for each instrument.

Codex Biosolutions, **Inc**. 401 Professional Drive, Suite 160, Gaithersburg, MD 20879
Tel: (240)-632-8810; Fax: (240)-632-8820 E-Mail: orders@codexbiosolutions.com
http://www.codexbiosolutions.com/

Appendix

(**A**)



(**B**)

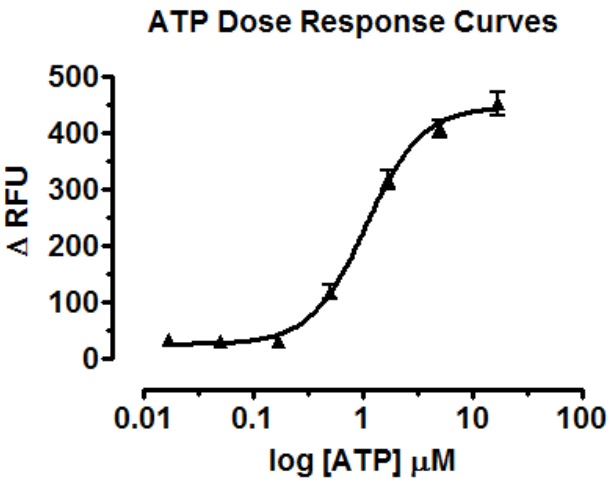


Figure 1. Response of endogenous P2Y receptors to ATP. HEK293 cells were plated overnight in 20 µl culture medium on a 384 well Biocoat poly-D lysine coated plate. The next day, the cells were dye-loaded by adding 20 µl of 2X Dye Loading Buffer and incubating for 1 hour at 37°C. ATP solution was added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

Limited Label License

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product for research use only by the buyer, where such research does not involve testing, analysis or screening services for any third party in return for compensation on a per test basis. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data to third parties; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For more information, contact Business Development, Codex Biosolutions, Inc. 401 Professional Drive, Suite 160, Gaithersburg, MD 20878, USA. Tel: (240)-632-8810. Fax: (240)-632-8820

Codex Biosolutions, **Inc**. 401 Professional Drive, Suite 160, Gaithersburg, MD 20879
Tel: (240)-632-8810; Fax: (240)-632-8820 E-Mail: orders@codexbiosolutions.com
http://www.codexbiosolutions.com/