BD™

Technical Data Sheet

Calcium Assay Kit

Product Information

Catalog Number:	**640176**
Size	**Reagents for 10 plates**
Components:	**Calcium Indicator, 1 vial, lyophilized**
	10X Signal Enhancer, 10 ml
	1X Calcium Assay Buffer, 90 ml

Description

The BD™ Calcium Assay Kit allows homogeneous measurement of intracellular calcium changes caused by activation of G-protein coupled receptors or calcium channels. The assay involves only one dye addition step and does not require washing, allowing easy implementation in a high throughput environment.

Storage

Calcium Indicator should be protected from light and stored at -20˚C. 10X Signal Enhancer and Calcium Assay Buffer should be stored at room temperature.

Materials not included

100% DMSO	Sigma D4540
Probenecid	Sigma P8761

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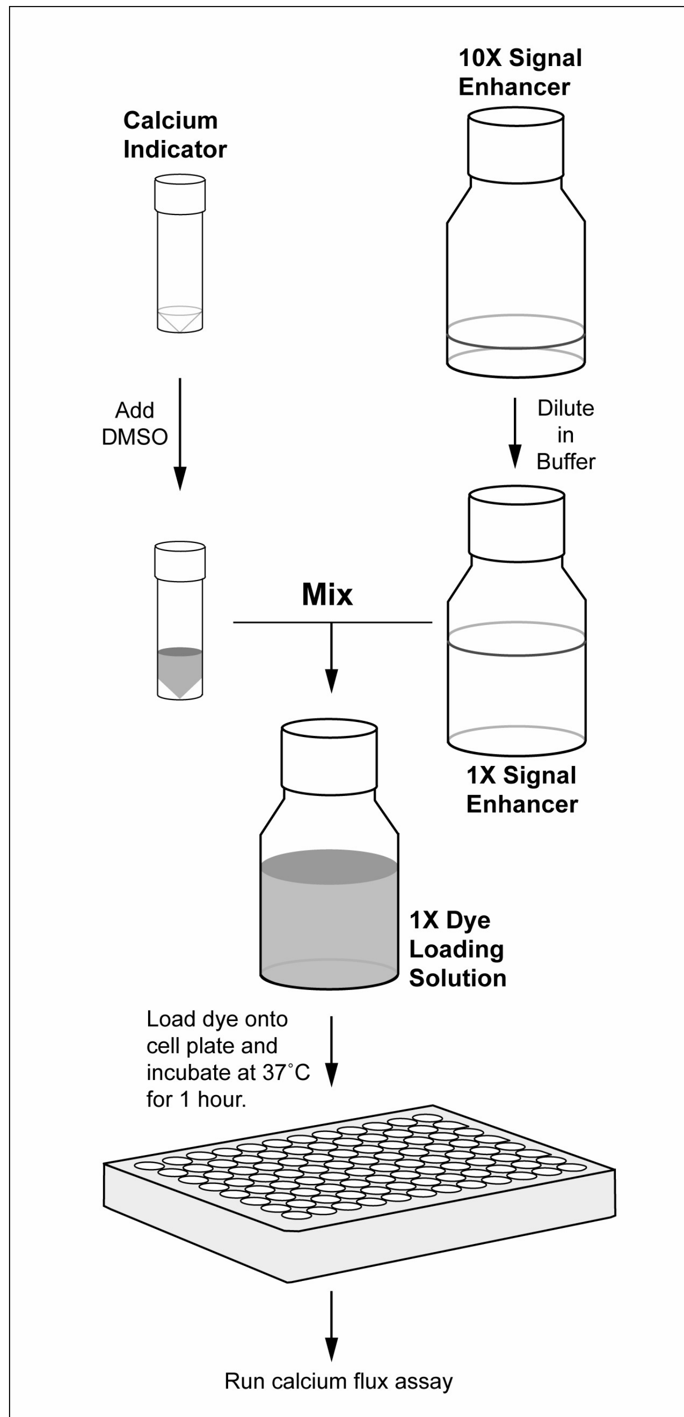
CALCIUM ASSAY FLOW CHART



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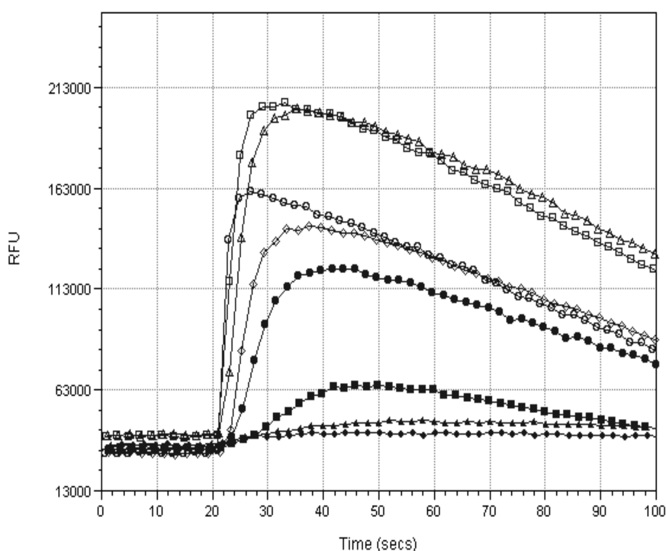
DATA EXAMPLES

A **B**



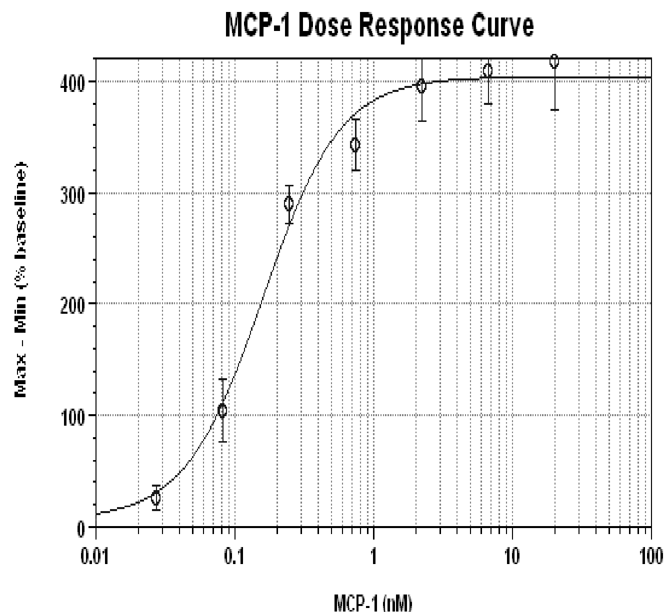


Figure 1. Response of Chemokine Receptor 2 (CCR2) to human monocyte chemoattractant protein-1 (MCP-1). HEK293 cells stably transfected with Gαqi and CCR2 genes were plated overnight in 100 μl culture medium on a 96 well Biocoat poly-D lysine coated plate. The next day, the cells were dye-loaded by adding 100 μl 1X Dye-loading solution (BD™ Calcium Assay Kit) and incubating for 1 hour at 37˚C. MCP-1 was added (50 μl/well) by a FlexStation (Molecular Devices), and the data was recorded simultaneously. **A**. Kinetic curve of calcium response to different concentrations of MCP-1. **B.** MCP-1 dose response curve (n = 4). EC50 = 0.159 nM.

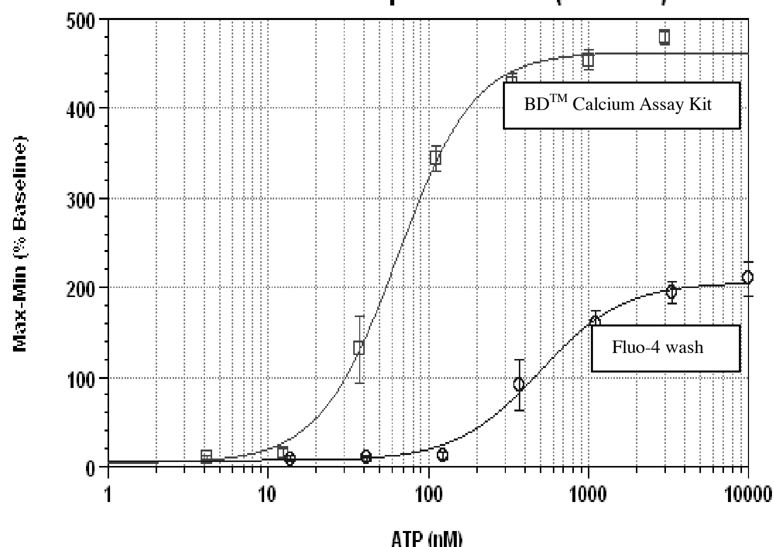


Figure 2. Response of CHO-K1 endogenous P2Y receptor to ATP. CHO-K1 cells were plated overnight in 100 μl culture medium on two 96 well plates. The next day, One plate of cells was dye-loaded with 100 μl/well of 1X Dye-loading solution (BD™ Calcium Assay Kit), and the other one was loaded with 100 μl/well of Fluo-4. After 1 hour of incubation at 37˚C, cells loaded with Fluo-4 were washed three times with HBSS-Hepes buffer. The same amount of ATP was added to both plates by a FlexStation (Molecular Devices), and the data was recorded simultaneously.
EC50 of ATP using BD™ Calcium Assay Kit is 64 nM.
EC50 of ATP using Fluo-4 wash method is 497 nM.

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CALCIUM FLUX ASSAY PROTOCOL

Note. Please finish reading the whole protocol before you start the experiment.

CELL PREPARATION

Cell number needs to be optimized for each assay. Optimal assay conditions require a confluent monolayer of cells prior to the assay.

- For adherent cells, plate 50K to 70K cells/well for a 96 well plate and 12K to 20K cells/well for a 384 well plate the day before the experiment. Add 100 µl/well of cell suspension to 96-well plates or 25 µl/well to 384-well plates, allow cells to attach and grow overnight for 16 to 24 hours in cell culture incubators. Prior to dye loading the plate, examine the cells for confluency and overall health.
- For non-adherent cells, dispense 100 µl/well of cells in culture medium to 96-well or 25 µl/well to 384-well poly-D Lysine or other ECM coated plates a couple of hours before the experiment. Allow cells to settle and attach to the bottom of the plates at room temperature. Centrifuge briefly with brake off prior to the experiment.

PREPARATION of 1X DYE-LOADING SOLUTION

1. Remove a vial of Calcium Indicator from -20˚C, and allow to sit at room temperature for 5 minutes to warm up. Add 100 µl 100% DMSO, reconstitute the pellet by pipetting up and down several times.

Note. To completely solublize the Calcium Indicator, keep the reconstituted Calcium Indicator at room temperature for 10 minutes before the preparation of 1X Dye-loading solution.

2. To prepare 1X Signal Enhancer, transfer 10 ml of 10X Signal Enhancer to 90 ml of 1X Calcium Assay buffer, mix well.

Note. For cells that require probenecid for loading (e.g. CHO), prepare fresh probenecid stock in 1N NaOH and then dilute the stock in 1X Signal Enhancer to a concentration of 1 mM to 5 mM.

3. To prepare 1X Dye-loading Solution for one cell plate, pipette 10 ml of 1X Signal Enhancer to a separate tube, add 10 µl of Calcium Indicator to 10 ml of 1X Signal Enhancer, mix by inverting the tube several times.

4. Aliquot the unused Calcium Indicator to several microfuge tubes, seal tightly and store at -20°C protected from light. 1X Signal Enhancer could be stored at room temperature.

Note. The reconstituted Calcium Indicator is stable for at least one month if the tube is sealed tightly. For best result, place the tubes in a zip lock plastic bag with desiccant and avoid repetitive freeze-thaw cycles.

DYE LOADING

1. Remove cell plates from incubator and add an equal volume of 1 X Dye-loading Solution to each well (e.g. 100 µl to 100 µl culture medium/well for 96-well plates, or 25 µl to 25 µl culture medium/well for 384-well plates).

Note. The components of the kit have no interference with serum or phenol red. However, for some assays that require a serum free environment, culture medium that contains serum should be removed prior to dye loading and replaced with an equal volume of 0.5X Dye-loading Solution.
The dye-loading solution is stable for 8 to 12 hours at room temperature and 2-3 weeks at -20C.

2. Incubate cell plates with dye for 1 hour in cell culture incubator.

Note. If the calcium flux assay is going to be performed at room temperature, place the cell plates at room temperature for at least 20 minutes to cool down before placing the plates on readers. If the calcium flux assay requires 37°C, perform the assay immediately after dye-loading.

CALCIUM FLUX ASSAY

Place the cell plates on a FLIPR, FlexStation or FDSS, and perform calcium flux assay as described in instrumentation manuals.

For assays performed on a FlexStation, use the following wavelength parameters:
Excitation: 485 nm
Emission: 525 nm
AutoCutoff: on (515 nm)

For assays performed on a FLIPR and an FDSS, use the standard filters for calcium assays.

Note. Dispense speed and height for compound additions need to be optimized for each assay. In general, a dispense speed higher than the settings for other calcium flux assays is recommended.

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TROUBLESHOOTING GUIDE

1. **Low baseline fluorescent signal**
   - Inspect the cell density and morphology under a microscope. Low cell density or unhealthy cells could result in low baseline signal.
   - Check the storage of Calcium Indicator. Calcium Indicator needs to be stored in a tightly sealed tube frozen with desiccant. Once it is reconstituted, repetitive freeze thaw cycles should be avoided.
   - Make sure that Calcium Indicator is mixed well with 1X Signal Enhancer before dye loading.

2. **Response to agonist lower than expected**
   - Check the overall health of cells.
   - Cell density is too high or too low. Cell number titration may be necessary.

3. **Well-to-well variations after agonist addition.**
   - Optimize the dispense height and speed for ligand additions to ensure instant mixing.
   - Cells should be evenly distributed among wells. Before plating, microscopically examine the culture to be sure that they have been broken up into single cells. Clumpy cells respond with greater variability.
   - Check liquid handling system for accuracy of dispense.

4. **Response from cells after buffer addition.**
   - Unhealthy cells could respond to buffer addition. Make sure cells are not over confluent or unhealthy before plating.
   - Cells are disturbed by high compound addition speed. Set the pipetting speed and height properly to avoid stimulating the cells by physical forces during compound addition.

5. **Response from cells after the addition of buffer containing only DMSO**
   - If the calcium flux experiment is conducted at room temperature, allow the cell plates to equilibrate at room temperature for a longer period of time (ie. one hour) after dye-loading at 37 degree.
   - Allow cells to adapt to a DMSO environment prior to drug addition by adding DMSO in Dye-loading Solution (slightly less than final concentration of DMSO after drug addition).

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