Technical Bulletin

Fluo-4 No Wash Calcium Assay Kit

Catalogue number MAK552

Product Description

The Fluo-4 No Wash Calcium Assay Kit provides a homogeneous fluorescence-based assay for detecting the intracellular calcium mobilization. Cells expressing a GPCR of interest that signals through calcium are pre-loaded with Fluo-4 AM which can cross the cell membrane. Once inside the cell, the lipophilic blocking groups of Fluo-4 AM are cleaved by non-specific cell esterase, resulting in a negatively charged Fluo-4 dye that stays inside cells, and its fluorescence is greatly enhanced upon binding to calcium. When cells are stimulated with screening compounds, the receptor signals the release of intracellular calcium, which greatly increases the fluorescence of Fluo-4.

Components

The kit is sufficient for 10 fluorometric test plates.

- Fluo-4 AM 1 Vial
  Catalogue Number MAK552A
- 10X F127 Plus 10 mL
  Catalogue Number MAK552B
- Hanks' Buffer with 20 mM HEPES (HHBS) 100 mL
  Catalogue Number MAK552C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Fluorescence multiwell plate reader
- Black, flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5mL microcentrifuge tubes
- DMSO (Catalogue number D4540 or equivalent)

Precautions and Disclaimer

For R&D use only.Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at -20°C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate to room temperature prior to use.

Procedure

All samples and standards should be run induplicate.

Preparation of Stock Solutions

Fluo-4 AM Stock Solution: Add 200 µL of DMSO into the vial of Fluo-4 AM and mix well. 20 µL of Fluo-4 Stock Solution is enough for one 96-well plate.

Assay Buffer (1X): Make 1X Assay Buffer by adding 1 mL of 10X F127 Plus into 9 mL of HHBS buffer and mix well. 10 mL of 1X Assay Buffer is enough for one 96-well plate.

Note: Store all the unused stock solutions at -20°C, protected from light. Avoid repeated freeze-thaw cycles.

Preparation of Working Solution

Fluo-4 AM Dye-Loading Solution: Add 20 µL of Fluo-4 AM Stock Solution into 10 mL of 1X Assay Buffer and mix well. This working solution is stable for at least 2 hours at room temperature.

Sample Preparation

Adherent Cells:

Plate cells overnight in growth medium at 40,000 to 80,000 cells/well/100 µL for a 96-well plate or 10,000 to 20,000 cells/well/25 µL for a 384-well plate.

Non-adherent Cells:

Centrifuge the cells from the culture medium and then suspend the cell pellet in cell growth medium or HHBS at 125,000 to 250,000 cells/well/100 µL for a 96-well poly-D lysine plate or 30,000 to 60,000 cells/well/25 µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.

Assay Reaction

1. Add 100 µL of Fluo-4 AM dye loading solution into the 96-well cell plate. Use 25 µL/well if using 384-well plates.
2. Incubate the dye-loading plate in a cell incubator for 1 hour, and then incubate the plate at room temperature for another 15 to 30 minutes.

Note: If the assay requires 37°C, perform the experiment immediately without further room temperature incubation.

3. Prepare the compound plate with HHBS or desired buffer.

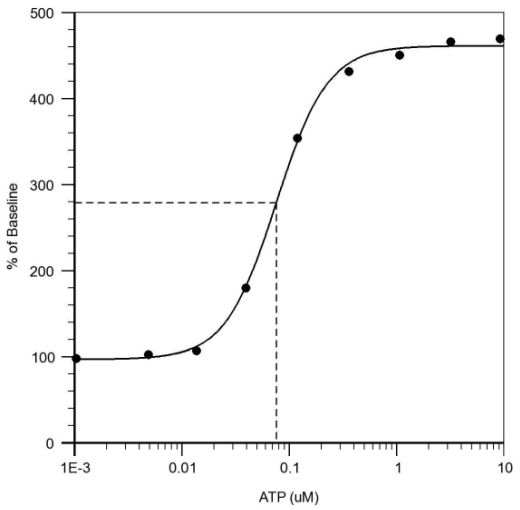
Measurement

Run the calcium flux assay by monitoring the fluorescence intensity at λEx/λEm = 490/525 nm.

Results

1. The reading (% of Baseline) obtained from the blank tandard well is used as a negative control.
2. Subtract this value from the other Standards' readings to obtain the base-line corrected values.
3. Plot the standards' readings to obtain a Standard curve and equation.
4. The concentration of the test samples may be determined from the Standard curve.

Figure 1.
Example of Experimental Data:



ATP dose response was measured in CHO-K1 cells with Fluo-4 No Wash Calcium Assay Kit. CHO-K1 cells were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of dye-loading solution using the Fluo-4 No Wash Calcium Assay Kit for 1 hour at room temperature. ATP (50µL/well) was added by Flexstation 3 (MDC) to achieve the final indicated concentrations.

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