Cell Meter™ No Wash and Probenecid-Free Endpoint Calcium Assay Kit *Optimized for microplate reader*

Catalog number: 36312
Unit size: 100 Tests

Component	Storage	Amount
Component A: Fluo-8E™ AM	Freeze (<-15 °C), Minimize light exposure	2 vials, lyophilized
Component B: 10X Pluronic® F127 Plus	Freeze (<-15 °C), Minimize light exposure	1 bottle (1 mL)
Component C: HHBS (Hanks' with 20 mM Hepes)	Refrigerate (2-8 °C)	1 bottle (20 mL)

OVERVIEW

Cell Meter™ No Wash and Probenecid-Free Endpoint Calcium Assay Kit enables homogeneous fluorescence-based assays for detecting intracellular calcium mobilization without the need to use kinetics reading mode. It can be used on Fluorescence microplate readers with bottom read mode that do not have a build-in liquid dispenser or the ability of kinetics reading. After loading the Fluo-8E™AM dye into cells of interest, without wash steps, one can simply add the calcium flux agonist by a liquid dispenser or hand pipetting, and then read the plate by a fluorescent reader. Fluo-8E™ AM can cross cell membrane passively by diffusion. Once inside the cells, the lipophilic blocking groups of Fluo-8E™ AM are cleaved by esterase, resulting in a negatively charged fluorescent dye that stays inside cells. Its fluorescence is greatly enhanced and long lasting upon binding to calcium. When cells expressing GPCR of interest are stimulated with an agonist, the receptor signals the release of intracellular calcium, which significantly increases the fluorescence of Fluo-8E™. The characteristics of its high sensitivity, >100 times fluorescence enhancement and long lasting fluorescent signal make Fluo-8E™ an ideal indicator for the measurement of cellular calcium on fluorescence microplate readers that do not have the fluid transfer and kinetic reading mode capability. The Cell Meter™ No Wash and Probenecid-Free Endpoint Calcium Assay Kit can be performed in a 96-well or 384-well microtiter-plate format.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Add Fluo-8E™ AM dye-loading solution (100 µL/well for 96-well plate or 25 µL/well for 384-well plate)
3. Incubate at 37°C for 60 minutes
4. Add 50 µL calcium flux stimulator
5. Monitor fluorescence intensity at Ex/Em = 490/525 nm

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Recommended plate:	Black wall/Clear bottom
Instrument specification(s):	Bottom read mode

Other Instruments: FLIPR, NOVOStar, FlexStation, ViewLux, IN Cell Analyzer, ArrayScan, FDSS

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Fluo-8E™ AM stock solution:
Add 10 µL of DMSO into the vial of Fluo-8E™ AM (Component A) and mix well.

Note 10 µL of Fluo-8E™ AM stock solution is enough for 50 assays (Half of the 96-well plate).

Note Unused Fluo-8E™ AM stock solution can be aliquoted and stored at < -20 °C for more than one month if the tubes are sealed tightly. Protect from light and avoid repeated freeze-thaw cycles.

2. Assay Buffer (1X):
Add 9 mL of HHBS (Component C) into 10X Pluronic® F127 Plus (1 mL, Component B), and mix them well.

Note 10 mL of Assay Buffer (1X) is enough for one plate. Aliquot and store unused 1X assay buffer at < -20 °C. Protect from light and avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

Fluo-8E™ AM dye-loading solution:
Add 10 µL of Fluo-8E™ AM stock solution into 5 mL of Assay Buffer (1X), and mix them well.

Note This working solution is stable for at least 2 hours at room temperature.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Fluo-8E™ AM dye-loading solution into the cell plate. Do not remove the growth medium from the cell plate.

2. Incubate the dye-loading plate in a 5% CO_2 incubator at 37°C for 45 - 60 minutes.

3. Prepare the Calcium stimulator solution (5X) with HHBS or your desired buffer.

4. Add 50 µL of the prepared stimulator and run the calcium flux assay immediately by monitoring the fluorescence intensity at Ex/Em = 490/525 nm (Cutoff=515 nm) with bottom read mode.

   Note To achieve the best results, it is important to run the assay within 1 minute after the addition of the agonist. It is also important to make sure the time between the agonist addition and the beginning of the actual reading stays constant for all the samples.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate ATP samples. We

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recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

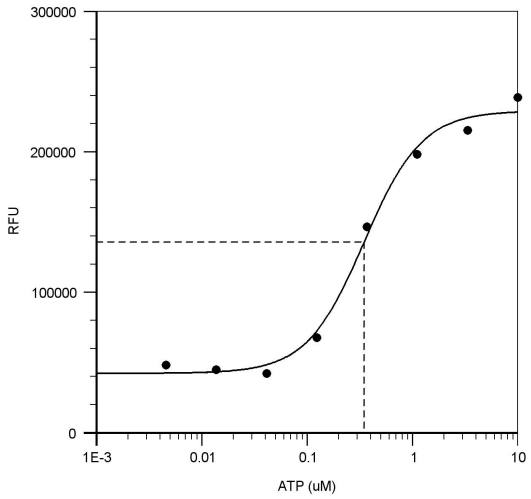

Figure 1. The ATP dose dependent intracellular calcium release was measured by Cell Meter™ No Wash and Probenecid-Free Endpoint Calcium Assay Kit in CHO-K1 cells in a 96-well plate. 3 columns of cells were incubated with Fluo-8E™ AM dye loading solution for 1 hr at 37°C before ATP was added into all 3 columns of the wells. The plate was read immediately after the addition of the ATP by ClarioStar (BMG Labtech) at Ex/Em = 490/525 nm with bottom and endpoint read mode.