Screen Quest™ Fluo-4 No Wash Calcium Assay Kit

Catalog number: 36325, 36326
Unit size: 10 Plates, 100 Plates

Component	Storage	Amount	
		Cat No. 36325	Cat No. 36326
Component A: Fluo-4 AM	Freeze (<-15 °C), Minimize light exposure	1 vial, lyophilized	10 vials, lyophilized
Component B: 10X Pluronic® F127 Plus	Freeze (<-15 °C), Minimize light exposure	1 bottle (10 ml/bottle)	10 bottles (10 mL/bottle)
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	Freeze (<-15 °C), Minimize light exposure	1 bottle (100 mL)	Not included

OVERVIEW

Calcium flux assays are preferred methods in drug discovery for screening G protein coupled receptors (GPCR). Screen Quest™ 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 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 stimulated with screening compounds, the receptor signals release of intracellular calcium, which greatly increase the fluorescence of Fluo-4. This Screen Quest Fluo-4 No Wash Calcium Assay Kit provides an optimized assay method for monitoring G-protein-coupled receptors (GPCRs) and calcium channels. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Add Fluo-4 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 1 hour
4. Monitor fluorescence intensity at Ex/Em = 490/525 nm

Important Do not add additional probenecid. Thaw all the kit components at room temperature before use.

KEY PARAMETERS

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

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-4 AM stock solution:
Add 200 µL of DMSO into the vial of Fluo-4 AM (Component A), and mix them well.

Note 20 µL of Fluo-4 NW stock solution is enough for one plate. Unused Fluo-4 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):
Make 1X Assay Buffer by adding 1 mL of 10X Pluronic® F127 Plus (10 mL, Component B) into 9 mL of HHBS buffer (Component C) and mix well.

Note 10 mL of 1X assay buffer is enough for one plate. Aliquot and store unused 10X Pluronic® F127 Plus (Component B) at < -20 °C. Protect from light and avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

Fluo-4 AM dye-loading solution:
Add 20 µL of Fluo-4 NW 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.

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-4 AM dye-loading solution into the cell plate.

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 your desired buffer.

4. Run the calcium flux assay by monitoring the fluorescence intensity at Ex/Em = 490/525 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (% of Baseline) 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 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

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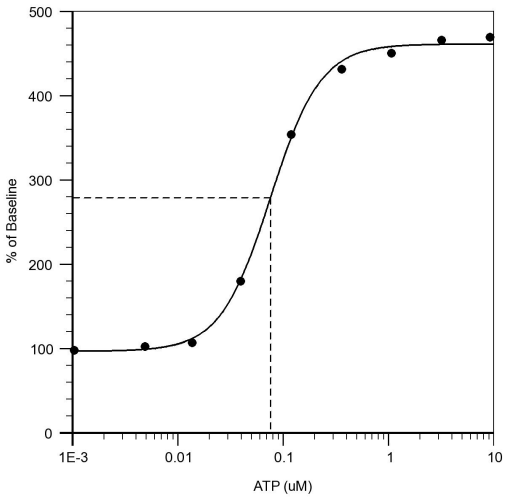

Figure 1. ATP Dose Response was measured in CHO-K1 cells with Screen Quest™ Fluo-4 No Wash Calcium Assay Kit. CHO-K1 cells were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of dye-loading solution using the Screen Quest™ 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.

DISCLAIMER