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)	Amount (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

Screen Quest™ Fluo-4 No Wash Calcium Assay Kit is designed for fluorescence-based detection of intracellular calcium mobilization in live cells. Calcium flux assays are commonly used in drug discovery for screening G protein-coupled receptors (GPCRs) and calcium channels. The kit uses Fluo-4 AM, a cell-permeant calcium indicator that enters cells and is converted by intracellular esterases into the negatively charged Fluo-4 dye retained inside cells. When cells are stimulated with screening compounds, receptor-mediated calcium release or calcium influx increases the fluorescence of Fluo-4 upon calcium binding. The no-wash homogeneous assay format simplifies calcium flux detection and supports microplate-based workflows in 96-well or 384-well formats that can be adapted to automation. Screen Quest™ Fluo-4 No Wash Calcium Assay Kit is suitable for GPCR calcium signaling studies, calcium channel assays, intracellular calcium mobilization analysis, and high-throughput calcium screening applications.

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 Note

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

KEY PARAMETERS

Fluorescence microplate reader

Excitation	490 nm
Emission	525 nm
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

CELL PREPARATION

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

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

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.

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 un-used 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.

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

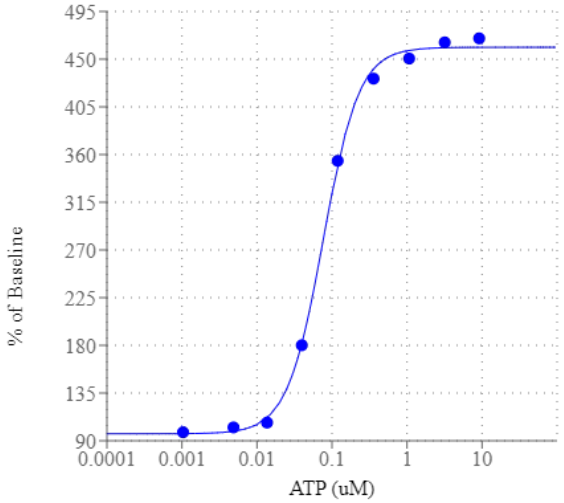

Figure 1. ATP Dose Response was measured in CHO-K1 cells with Screen Quest™ Fluo-4 No Wash Calcium Assay Kit. CHO-K1 cells were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of dye-loading solution using the Screen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

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