Screen Quest™ Fluo-8 Medium Removal Calcium Assay Kit *Optimized for Difficult Cell Lines*

Catalog number: 36307, 36308, 36309
Unit size: 1 Plate, 10 Plates, 100 Plates

Component	Storage	Amount		
		Cat No. 36307	Cat No. 36308	Cat No. 36309
Component A: Fluo-8 NW	Freeze (<-15 °C), Minimize light exposure	1 vial, lyophilized	1 vial, lyophilized	10 vials, lyophilized
Component B: 10X Pluronic® F127 Plus	Freeze (<-15 °C), Minimize light exposure	1 bottle (1 mL)	10 bottles (1 mL/bottle)	10 bottles (10 mL/bottle)
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	Freeze (<-15 °C), Minimize light exposure	1 bottle (9 mL)	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-8 NW 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 our proprietary Fluo-8 NW which can cross cell membrane. Fluo-8 NW is the brightest calcium indicator available for HTS screening. Once inside the cell, the lipophilic blocking groups of Fluo-8 NW are cleaved by non-specific cell esterase, resulting in a negatively charged fluorescent 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-8 NW. The characteristics of its long wavelength, high sensitivity, and 100-250 times fluorescence increases (when it forms complexes with calcium) make Fluo-8 NW an ideal indicator for measurement of cellular calcium. This Screen Quest Fluo-8 NW 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
2. Remove the growth medium
3. Add Fluo-8 NW dye working solution
4. Incubate at RT for 1 hour
5. Monitor fluorescence intensity at Ex/Em = 490/525 nm

Important Do not add additional probenecid. It is recommended to incubate the dye working solution no longer than 2 hours.
Thaw all components to room temperature before beginning protocol.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	510 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-8 NW stock solution:
For Cat No. 36307, add 10 µL of DMSO into Fluo-8 NW (Component A), and mix them well.
For Cat No. 36308 and 36309, add 100 µL of DMSO into Fluo-8 NW (Component A), and mix them well.

Note 10 µL of Fluo-8 NW stock solution is enough for 1 plate.

2. Assay Buffer stock solution (1X):
For Cat No. 36307 and 36308, add 9 mL of HHBS (Component C) into 10X Pluronic® F127 Plus (1 mL, Component B) and mix well.
For Cat No. 36309, add the whole bottle of 10X Pluronic® F127 Plus (10 mL, Component B) into 90 mL of HHBS buffer (not included in kit) and mix well.

Note 10 mL of 1X Assay Buffer is enough for one plate.

PREPARATION OF WORKING SOLUTION

Add 10 µL of Fluo-8 NW DMSO 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. Remove the growth medium from the cell plate.

   Note It is important to remove the growth medium in order to minimize background fluorescence and compound interference with serum or culture media.

   Note Alternatively, grow the cells in growth medium with 0.5% - to 1% FBS to avoid medium removal step. In this case, 2X dye loading solution in HHBS buffer is needed. [We offer 2 separate no wash calcium assay kits (Cat No. 36315 and Cat No. 36316) for those who use 0.5% to 1% FBS in growth medium to avoid the medium removal step].

2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Fluo-8 NW dye working solution into the cell plate.

3. Incubate the dye-loading plate in a cell incubator for 30 minutes, and then incubate the plate at room temperature for another 30 minutes.

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

   Note If the cells can function well at room temperature for longer time, incubate the cell plate at room temperature for 1 - 2 hours (It is recommended that the incubation time be no longer than 2 hours.)

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

4. Prepare the compound plates with HHBS or your desired buffer.

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

Note It is important to run the signal test before your experiment. Different instruments have their own intensity range. Adjust the signal test intensity to the level of 10% to 15% of the maximum intensity counts. For example, the maximum fluorescence intensity count for FLIPR-384 is 65,000, so the instrument setting should be adjusted to have its signal test intensity around 7,000 to 10,000.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU (Max-Min)) 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 Carbachol dose 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

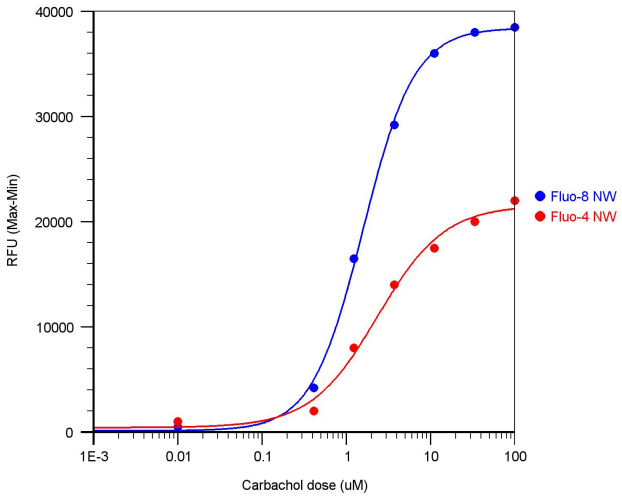

Figure 1. Carbachol Dose Response was measured in HEK-293 cells with Screen Quest™ Fluo-8 NW Assay Kit and Fluo-4 NW Assay Kit. HEK-293 cells were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The growth medium was removed, and the cells were incubated with 100 µL of dye-loading solution using the Screen Quest™ Fluo 8-NW calcium assay kit or the Fluo-4 NW kit (according to the manufacturer's instructions) for 1 hour at room temperature. Carbachol (25µL/well) was added by NOVOstar (BMG Labtech) to achieve the final indicated concentrations. The EC50 of Carbachol using Fluo8 NW is about 1.2 µM.

DISCLAIMER