Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Medium*

Ordering Information:
Product Number: #36315 (10 plates), #36316 (100 plates)

Instrument Platform:
FLIPR, FDSS, NOVOStar, FlexStation, ViewLux, IN Cell Analyzer, ArrayScan

Storage Conditions:
Keep in freezer and avoid light.

Introduction

Calcium flux assays are preferred methods in drug discovery for screening G protein coupled receptors (GPCR). Screen Quest™ Fluo-8 NW Calcium Assay Kits provide homogeneous fluorescence-based assays for detecting intracellular calcium mobilization. Cells expressing a GPCR of interest that signals through calcium are pre-loaded with Fluo-8 NW which can cross cell membrane. Once inside the cell, the lipophilic blocking groups of Fluo-8 NW are cleaved by esterases, 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 agonists, the receptor signals the release of intracellular calcium, which greatly increase the fluorescence of Fluo-8 NW. The characteristics of its long wavelength, high sensitivity, and >100 times fluorescence enhancement (when it forms a complex with calcium) make Fluo-8 NW an ideal indicator for measurement of cellular calcium. The Screen Quest Fluo-8 NW Calcium Assay Kits provide 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.

Kit Key Features

Increased Signal Intensity:	Fluo-8 NW is the brightest calcium indicator, more than 2 fold brighter than Fluo-4 AM, and 4 times brighter than Fluo-3 AM.
Rapid Dye Loading:	Dye loading at RT (rather than 37 °C required for Fluo-4 AM)
Convenient and Robust:	Formulated to have minimal hands-on time. No wash step needed.
Versatile applications:	Compatible with many cell lines and targets without ligand or target interference.

Kit Components

Materials	#36315 (10 plates)	#36316 (100 plates)
Component A: Fluo-8 NW	1 vial, lyophilized	10 vials, lyophilized
Component B: 10X Pluronic F127 Plus	10 bottles (1 ml/bottle)	10 bottles (10 ml/bottle)
Component C: HHBS	1 bottle (100 ml)	Not included

Materials Required (but not provided)

- 96 or 384-well microplate: Tissue culture microplate with black wall and clear bottom.
- Fluorescence microplate readers with a filter set of Ex=490 nm/Em=520-530 nm.
- HHBS (1X Hank's with 20 mM Hepes buffer, pH 7.0).
- 100% DMSO.

Assay Protocol (for 1 plate)

Brief Summary

Prepare cells in growth medium with 0.5-1% FBS → Add Fluo-8 NW dye-loading solution (100 µL/well for 96-well-plate or 25 µL/well for 384-well-plate) → Incubate at room temperature for 1hr → Read Fluorescence at Ex=490/Em=520

Warning: No addition of probenecid is needed for using this kit

1. Prepare Cells

1.1 For adherent cells, plate cells overnight in growth medium with 0.5-1%FBS at 40,000 to 80,000 cells/well/100µl for 96-well or 10,000 to 20,000 cells/well/25µl for 384-well plates.

1.2 For non-adherent cells, centrifuge the cells from the culture medium and then suspend the cell pellets in equal amount of HHBS and Fluo-8 NW dye-loading solution (see steps 2.4 below) at 125,000 to 250,000 cells/well/100µl for 96-well or 30,000 to 60,000 cells/well/25µl for 384-well poly-D lysine plates. Centrifuge the plates at 800 rpm for 2 minutes with break off prior to the experiments
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for the intracellular calcium mobilization.

2. Prepare Fluo-8 NW dye-loading solution (for 1 plate)

2.1 Thaw 1 vial of Component A (Fluo-8 NW), 1 bottle of Component B (10X Pluronic F127 Plus) and Component C (HHBS) at room temperature before use.

2.2 Make Fluo-8 NW stock solution by adding 200 µl DMSO into component A (Fluo-8 NW) and mixing them well.
Note: 20 µl of reconstituted Fluo-8 NW is enough for 1 plate, un-used reconstituted *Fluo-8 NW can be aliquoted and stored at ≤ -20°C for more than one month if the tubes are sealed tightly, avoiding light and repeated freeze-thaw cycles.*

2.3 Make 1X assay buffer
a). For **Cat# 36315 (10 plates kit)**, make 1X assay buffer by adding **9 ml** component C **(**HHBS) into component B (10X Pluronic F127 Plus, 1 ml), mix them well.
b). For **Cat# 36316 (100 plates kit)**, make 1X assay buffer by adding whole component B (10 X Pluronic F127 Plus, 10 ml) into **90 ml** HHBS buffer (not included in the kit), mix them well.
Note: 10 ml 1X assay buffer is enough for 1 plate, aliquot and store un-used 1X assay buffer at ≤ -20°C, avoid light and repeated freeze-thaw cycles.

2.4 Make Fluo-8 NW dye-loading solution for one cell plate by adding 20 µl of DMSO reconstituted Fluo-8 NW (from step 2.2) into 10 ml of 1X assay buffer (from step 2.3), mixing them well. This working solution is stable for at least 2 hours at room temperature.

3. Run Calcium Assay

3.1 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) Fluo-8 NW dye-loading solution into the cell plate.
Note: Alternatively, one can grow the cells in growth medium with 5-to 10% FBS to improve cell growth. In this case, it is important to replace the growth medium with HHBS buffer in order to minimize background fluorescence, and compound interference with serum. (We offer 2 separate no wash calcium assay kits (#36308 and 36309) for people who prefer the medium removal step).

3.2 Incubate the dye-loading plate at cell incubator for 30 minutes, and then incubate the plate at room temperature for another 30 minutes.
Note 1: if the assay requires 37°C, perform the experiment immediately without further room temperature incubation.
Note 2: if the cells can be tolerated at room temperature for longer time, incubate the cell plate at room temperature for 1-2 hours.

3.3 Prepare the compound plates by using HHBS or your desired buffer.

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

Data Analysis

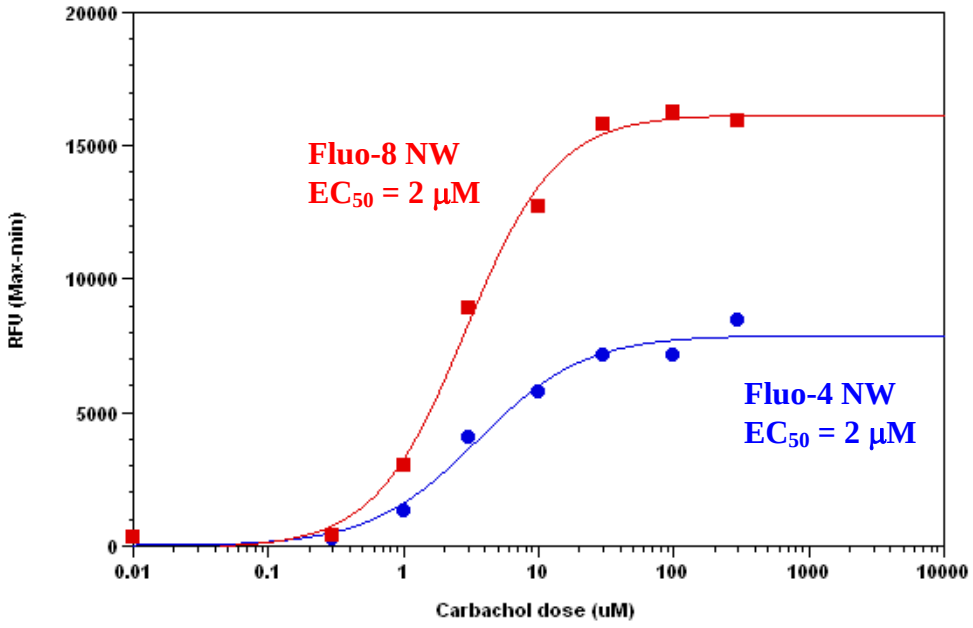

Figure 1. **Carbachol Dose Response in HEK-293 cells measured with Screen Quest™ Fluo-8 NW Calcium Assay Kit and Fluo-4 NW Calcium Assay Kit.** HEK-293 cells were seeded overnight in 40,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. The cells were incubated with 100 µl of the Screen Quest™ Fluo 8-NW calcium assay kit, or Fluo-4 NW kit (According to the Manufacture's instructions) for 1 hour at room temperature. Carbachol (50µl/well) was added by NOVOstar (BMG LabTech) to achieve the final indicated concentrations.

Warning: This kit is only sold for the end users. It is covered by a pending patent. Neither resale nor transfer to a third party is allowed without written permission from ABD Bioquest. Chemical analysis of kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@abdbioquest.com if you have any questions.

Related Products

21013	Fluo-3, AM *Custom packaging*	20x50 µg
21011	Fluo-3, AM *UltraPure grade*	1 mg
21021	Fura-2, AM *UltraPure Grade*	1 mg
21036	Indo-1, AM *Custom packaging*	20x50 µg
21032	Indo-1, AM *UltraPure Grade*	1 mg
20053	Pluronic® F-127 *10% solution in water*	10 mL
20052	Pluronic® F-127 *20% solution in DMSO*	10 mL
20060	Probenecid *Cell culture tested*	10x150 mg
20061	Probenecid *Water-soluble*	10x150 mg
21062	Rhod-2, AM *UltraPure Grade*	1 mg
21063	Rhod-2, AM *UltraPure Grade* *Bulk packaging*	50 mg
21064	Rhod-2, AM *UltraPure Grade* *Custom packaging*	20x50 µg
21070	Rhod-5N, AM	1 mg
21080	Quest Fluo-8™ AM *Cell-permeable*	1 mg
21081	Quest Fluo-8™, AM *Cell-permeable*	5x50 µg
21082	Quest Fluo-8™, AM *Cell-permeable*	10x50 µg
21083	Quest Fluo-8™, AM *Cell-permeable*	20x50 µg
21090	Quest Fluo-8H™, AM *Cell-permeable*	1 mg
21091	Quest Fluo-8H™, AM *Cell-permeable*	10x50 µg
21096	Quest Fluo-8L™, AM *Cell-permeable*	1 mg
21097	Quest Fluo-8L™, AM *Cell-permeable*	10x50 µg
21120	Quest Rhod-4™, AM	1 mg
21121	Quest Rhod-4™, AM	5x50 ug
21122	Quest Rhod-4™, AM	10x50 ug
21123	Quest Rhod-4™, AM	20x50 µg
36301	Screen Quest™ 10X calcium assay buffer	10 Plates
36302	Screen Quest™ 10X calcium assay buffer	100 Plates
36303	Screen Quest™ 10X calcium assay buffer with Phenol Red Plus™	10 Plates
36305	Screen Quest™ Coelenterazine Calcium Assay Kit *10 Plates*	1 kit
36306	Screen Quest™ Coelenterazine Calcium Assay Kit *10X10 Plates*	1 kit
36307	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal*	1 Plate
36308	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal*	10 Plates
36309	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal*	100 Plates
36314	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium*	1 Plate
36315	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium *	10 Plates
36316	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium*	100 Plates
36330	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal*	1 Plate
36331	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal*	10 Plates
36332	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal*	100 Plates
36333	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium*	1 Plate
36334	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium *	10 Plates
36335	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium*	100 Plates
2450	Trypan Blue, sodium salt *Cell culture tested*	100 g
2456	Trypan Red Plus™, sodium salt *0.1 M aqueous solution*	10 mL
2457	Trypan Red Plus™, sodium salt *0.1 M aqueous solution*	100 mL
2455	Trypan UltraBlue™, sodium salt	1 g