Screen Quest™ Fluo-8 No Wash Calcium Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36315 (10 plates), 36316 (100 plates)	Keep in freezer and avoid light	FLIPR, FDSS, NOVOStar,FlexStation, ViewLux, IN Cell Analyzer, ArrayScan

Introduction

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. Its fluorescence is greatly enhanced upon binding to calcium. When cells stimulated with agonists, the receptor signals the release of intracellular calcium, which significantly increase the fluorescence of Fluo-8 NW. The characteristics of its long wavelength, high sensitivity, and >100 times fluorescence enhancement make Fluo-8 NW an ideal indicator for the measurement of cellular calcium. The Screen Quest™ Fluo-8 NW Calcium Assay Kits provide an optimized assay method for monitoring the G-protein-coupled receptors 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.
Flexible Dye Loading:	Dye loading at RT (rather than 37 °C, which is required for Fluo-4 AM).
Convenient:	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

Components	**Amount**	
	Cat. # 36315 (10 plates)	**Cat. # 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 (Hanks' Buffer with 20 mM Hepes)	1 bottle (100 mL)	Not included

Assay Protocol for One Plate

Brief Summary

Prepare cells in growth medium with 0.5-1% FBS → Add Fluo-8 NW dye-loading solution (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Incubate at RT for 1 hour → Monitor fluorescence intensity at Ex/Em = 490/525 nm

Warning: Do not add additional probenecid.

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/100 µL for a 96-well plate or 10,000 to 20,000 cells/well/25 µL for a 384-well plate.

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellet in equal amount of HHBS and Fluo-8 NW dye-loading solution (see Step 2.4 below) at 125,000 to 250,000 cells/well/100 µL

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for a 96-well poly-D lysine plate or 30,000 to 60,000 cells/well/25 µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.
Note: Each cell line should be evaluated on the individual basis to determine the optimal cell density for the intracellular calcium mobilization.

2. Prepare Fluo-8 NW dye-loading solution:

2.1 Thaw all the kit components at room temperature before use.

2.2 Make Fluo-8 NW stock solution: Add 200 µL of DMSO into the vial of Fluo-8 NW (Component A), and mix them well.
Note: 20 µL of Fluo-8 NW stock solution is enough for one plate. Un-used Fluo-8 NW 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.

2.3 Make 1X assay buffer:
a). For **Cat. # 36315 (10 plates kit)**, make 1X assay buffer by adding **9 mL** of HHBS (Component C) into 10X Pluronic® F127 Plus (1 mL, Component B), and mix them well.

b). For **Cat. # 36316 (100 plates kit)**, make 1X assay buffer by adding the whole bottle of 10 X Pluronic® F127 Plus, (10 mL, Component B) into **90 mL** of HHBS buffer (not included in the kit), and mix them well.
Note: 10 mL of 1X assay buffer is enough for one plate. Aliquot and store un-used 1X assay buffer at ≤ -20 °C. Protect from light and avoid repeated freeze-thaw cycles.

2.4 Make Fluo-8 NW dye-loading solution for one cell plate: Add 20 µL of Fluo-8 NW stock solution (from Step 2.2) into 10 mL of 1X assay buffer (from Step 2.3), and mix 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) of Fluo-8 NW dye-loading solution (from Step 2.4) into the cell plate.
Note: Alternatively, grow the cells in growth medium with 5-10% 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 medium removal calcium assay kits (Cat.# 36308 and 36309) for those who prefer to keep the medium removal step].

3.2 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 1: If the assay requires 37 °C, perform the experiment immediately without further room temperature incubation.
Note 2: 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.)

3.3 Prepare the compound plate with HHBS or your desired buffer.

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

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Data Analysis

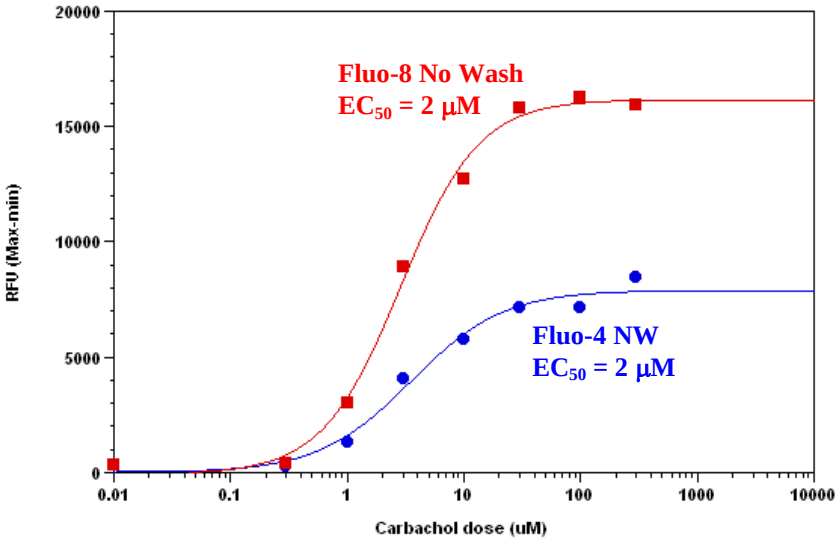

Figure 1. Carbachol Dose Response was measured in HEK-293 cells with Screen Quest™ Fluo-8 No Wash Calcium Assay Kit and Fluo-4 NW Calcium Assay Kit**.** HEK-293 cells were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of dye-loading solution using the Screen Quest™ Fluo-8 No Wash calcium assay kit or Fluo-4 NW kit (according to the manufacturer's instructions) for 1 hour at room temperature. Carbachol (50µL/well) was added by NOVOstar (BMG Labtech) to achieve the final indicated concentrations.

References

1. Falk, S and Rekling, J. C (2009) Neurons in the preBötzinger complex and VRG are located in proximity to arterioles in newborn mice. Neuroscience Letters. Volume 450, Issue 3, 229-234.
2. Ghoneum, M. Matsuur , M. and Gollapudi, S. (2009) An iron-based beverage, HydroFerrate fluid (MRN-100), alleviates oxidative stress in murine lymphocytes *in vitro*. Nutrition Journal **8**:18-23.
3. Satoru, T. Kobayashi, K. Takahashi, M. Katahira, K. Goryo, K. Matsushita, N. Yasumoto, K. Fujii-Kuriyama, Y. and Sogawa, K. (2009) Magnesium Deficiency Causes Loss of Response to Intermittent Hypoxia in Paraganglion Cells. J. Biol. Chem., Vol. 284, Issue 28, 19077-19089.

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