

Screen Quest™ Fluo-8 Medium Removal Calcium Assay Kit

<i>Ordering Information:</i>	<i>Storage Conditions:</i>	<i>Instrument Platform:</i>
Product Number: 36307 (1 plate), 36308 (10 plates), 36309(100 plates)	Keep in freezer Protect from light	FLIPR, FDSS, NOVOSTar, FlexStation ViewLux, IN Cell Analyzer, ArrayScan

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 esterase, 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 (when it forms a complex with calcium) 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 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. # 36307 (1 plate)	Cat. # 36308 (10 plates)	Cat. # 36309 (100 plates)
Component A: Fluo-8 NW	1 vial, lyophilized	1 vial, lyophilized	10 vials, lyophilized
Component B: 10X Pluronic® F127 Plus	1 bottle (1 mL)	10 bottles (1 mL/bottle)	10 bottles (10 mL/bottle)
Component C: HHBS (Hanks' with 20 mM HEPES)	1 bottle (9 mL)	1 bottle (100 mL)	Not included

Assay Protocol for One Plate

Brief Summary

Prepare cells → Remove the growth medium → 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 → Read fluorescence intensity at Ex/Em = 490/525 nm

Warning: Do not add additional probenecid. It is recommended to incubate the dye loading solution no longer than 2 hours.

1. Prepare cells:

- 1.1 For adherent cells: Plate cells overnight in growth medium at 40,000 to 80,000 cells/well/100 μ L for a 96-well plate or 10,000 to 20,000 cells/well/25 μ L 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 Fluo-8 NW dye-loading solution (see Step 2.4) at 125,000 to 250,000 cells/well/100 μ L for a 96-well poly-D lysine plate or 30,000 to 60,000 cells/well/ 25 μ L 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 an individual basis to determine the optimal cell density for the intracellular calcium mobilization.

2. Prepare Fluo-8 NW dye-loading solution (for one 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: Add 10 μ L (for **Cat. # 36307**) or 100 μ L (for **Cat. # 36308 and # 36309**) of DMSO into component A (Fluo-8 NW), and mix them well.
Note: 10 μ L of reconstituted Fluo-8 NW is enough for 1 plate. Unused Fluo-8 NW stock solution can be aliquoted and stored at $\leq -20^{\circ}\text{C}$ 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. # 36307 (1 plate kit) and 36308 (10 plates kit)**, make 1X assay buffer by adding **9 mL** of Component C (HHBS) into Component B (10X Pluronic® F127 Plus, 1 mL), and mix them well.
 - b). For **Cat. # 36309 (100 plates kit)**, make 1X assay buffer by adding the whole Component B (10X Pluronic® F127 Plus, 10 mL) 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 unused 1X assay buffer at $< -20^{\circ}\text{C}$. Protect from light and avoid repeated freeze-thaw cycles.
- 2.4 Make Fluo-8 NW dye-loading solution for one cell plate: Add 10 μ L of Fluo-8 NW DMSO 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 Remove the growth medium from the cell plate.
Note 1: It is important to remove the growth medium in order to minimize background fluorescence, and compound interference with serum or culture media.
Note 2: 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. # 36315 and Cat. # 36316) for those who use 0.5-to 1% FBS in growth medium to avoid the medium removal step].
- 3.2 Add 100 μ L/well (96-well plate) or 25 μ L/well (384-well plate) of Fluo-8 NW dye-loading solution (from Step 2.4) into the cell plate (from Step 3.1).
- 3.3 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 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.)
- 3.4 Prepare the compound plates with HHBS or your desired buffer.
- 3.5 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.

Data Analysis

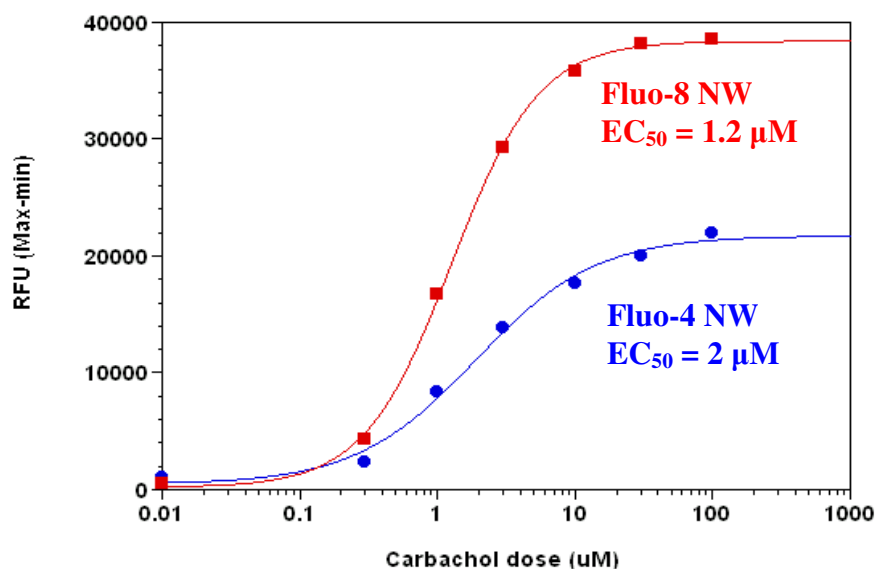


Figure 1. Carbachol Dose Response was measured in HEK-293 cells with Screen Quest™ Fluo-8 NW Assay kit and Fluo-4 NW Assay Kit. HEK-293 cells were seeded overnight at 40,000 cells/100 µL/well in a 96-well black wall/clear bottom costar plate. The growth medium was removed, and the cells were incubated 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 Fluo-8 NW is about 1.2 µM.

References

1. Falk, S and Rekling, J. C (2009) Neurons in the preBötzing complex and VRG are located in proximity to arterioles in newborn mice. *Neuroscience Letters*. Volume 450, Issue 3, 229-234.
2. Ghoneum, M. Matsuura, 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.