# Screen Quest™ Fluo-8 Medium Removal Calcium Assay Kit

| *Ordering Information* | *Storage Conditions* | *Instrument Platform* |
| --- | --- | --- |
| Product Number: 36307 (1 plate), 36308 (10 plates), 36309(100 plates) | Keep in freezer<br>Protect from light | FLIPR, FDSS, NOVOStar, FlexStation<br>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 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 make Fluo-8 NW an ideal indicator for the measurement of cellular calcium. The Screen Quest™ Fluo-8 NW Calcium Assay Kits 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 for1 hour → Monitor 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 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 Fluo-8 NW dye-loading solution (see Step 2.4) at 125,000 to 250,000 cells/well/100 µL for a 96-well poly-D lysine plate or 30,000 to 60,000 cells/well/ 25µL 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 Fluo-8 NW (Component A), 1 bottle of 10X Pluronic® F127 Plus (Component B) and HHBS (Component C) at room temperature before use.

2.2 Make Fluo-8 NW stock solution: Add 10 µL (for **Cat. # 36307**) or 100 µL (for **Cat. # 36308 and # 36309**) 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. Unused 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. # 36307 (1 plate kit)** and **36308 (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. # 36309 (100 plates kit)**, make 1X assay buffer by adding the whole bottle of 10X 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 unused 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 10 µL 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 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 (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

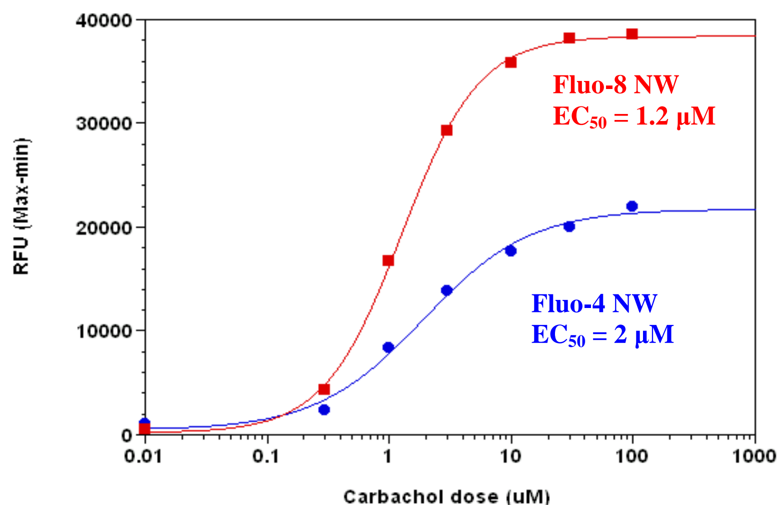


**Figure 1.** Carbachol Dose Response was measured in HEK-293 cells with Screen Quest™ Fluo-8 NW Assay Kit and Fluo-4 NW Assay Kit**.** HEK-293 cells were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The growth medium was removed, and the cells were 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 $EC_{50}$ of Fluo-8 NW is about 1.2 µM.

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## References


1. Emery Smith, Peter Chase, Colleen M. Niswender, Thomas J. Utley, Douglas J. Sheffler, Meredith J. Noetzel, Atin Lamsal, Michael R. Wood, P. Jeffrey Conn, Craig W. Lindsley, Franck Madoux, Mary Acosta, Louis Scampavia, Timothy Spicer, and Peter Hodder. Application of Parallel Multiparametric Cell-Based FLIPR Detection Assays for the Identification of Modulators of the Muscarinic Acetylcholine Receptor 4 (M4) *J Biomol Screen.* 2015; 20:858-868. doi:10.1177/1087057115581770
2. R. Benjamin Free, Lani S. Chun, Amy E. Moritz, Brittney N. Miller, Trevor B. Doyle, Jennie L. Conroy, Adrian Padron, Julie A. Meade, Jingbo Xiao, Xin Hu, Andrés E. Dulcey, Yang Han, Lihua Duan, Steve Titus, Melanie Bryant-Genevier, Elena Barnaeva, Marc Ferrer, Jonathan A. Javitch, Thijs Beuming, Lei Shi, Noel T. Southall, Juan J. Marugan, and David R. Sibley. Discovery and Characterization of a G Protein–Biased Agonist That Inhibits b- Arrestin Recruitment to the D2 Dopamine Receptor. *Mol Pharmacol* 86:96–105, July 2014, doi /10.1124/mol.113.090563
3. Annika Thorsell, Jenica D. Tapocik, Ke Liu, Michelle Zook, Lauren Bell, Meghan Flanigan, Samarjit Patnaik, Juan Marugan, Ruslan Damadzic, Seameen J. Dehdashti, Melanie L. Schwandt, Noel Southall, Christopher P. Austin, Robert Eskay, Roberto Ciccocioppo, Wei Zheng, and Markus Heilig. A Novel Brain Penetrant NPS Receptor Antagonist, NCGC00185684, Blocks Alcohol-Induced ERK-Phosphorylation in the Central Amygdala and Decreases Operant Alcohol Self-Administration in Rats. *J. Neurosci*., Jun 2013; 33: 10132 - 10142.
4. P. Senbagavalli, J. Nancy Hilda, V. D. Ramanathan, V. Kumaraswami, Thomas B. Nutman, and Subash Babu Immune Complexes Isolated from Patients with Pulmonary Tuberculosis Modulate the Activation and Function of Normal Granulocytes. *Clin Vaccine Immunol.,* Dec 2012; 19: 1965 - 1971.
5. Mikihito Kajiya, Isao Ichimonji, Christine Min, Tongbo Zhu, Jun-O Jin, Qing Yu, Soulafa A. Almazrooa, Seunghee Cha, and Toshihisa Kawai. Muscarinic Type 3 Receptor Induces Cytoprotective Signaling in Salivary Gland Cells through Epidermal Growth Factor Receptor Transactivation. *Mol. Pharmacol.,* 2012; 82: 115 - 124.
6. K. Ohta, N.J. Laborde, M. Kajiya, J. Shin, T. Zhu, A.K. Thondukolam, C. Min, N. Kamata, N.Y. Karimbux, P. Stashenko, and T. Kawai. Expression and Possible Immune-regulatory Function of Ghrelin in Oral Epithelium. *J. Dental Research* 2011; 90: 1286 - 1292.
7. Hasan M. S. Ibrahim, Mohammed I. Al-Salabi, Nasser El Sabbagh, Neils B. Quashie, Abdulsalam A. M. Alkhaldi, Roger Escale, Terry K. Smith, Henri J. Vial, and Harry P. de Koning. Symmetrical choline-derived dications display strong anti-kinetoplastid activity. *J. Antimicrob. Chemother.,* 2011; 66: 111 - 125.
8. 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.
9. 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.
10. 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.


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