



## Fluo-8 NW

Fluo-8 No Wash (NW) is the new generation of calcium assay dye for HTS applications

### Product Information

Product name cat.number	Packaging interest
<b>Fluo-8 NW Calcium Assay Kit, Medium removal</b> CJ256E, 1 plate CJ2560, 10 plates CJ2561, 100 plates	Remove the growth medium in order to minimize background fluorescence, and compound interference with serum or culture media.
<b>Fluo-8 NW Calcium Assay Kit, 1% FBS Medium</b> CJ255E, 1 plate CJ2550, 10 plates CJ2551, 100 plates	Grow the cells in growth medium with 0.5-to 1% FBS to avoid medium removal step
<b>Technical parameters</b>	$\lambda_{exc./em.}$ : 490 / 514 nm $K_d$ (calcium): 390 nM
<b>Instrument Plateform</b>	Twinkle LB 970, TriStar LB941, Mithras LB940, FLIPR, FDSS, NOVOSTar, FlexStation, ViewLux, IN Cell Analyzer, ArrayScan

**Storage:** -20°C. Protect from light.

### Introduction

Calcium flux assays are preferred methods in drug discovery for screening G protein coupled receptors (GPCR). Fluo-8 NW Calcium Assay Kits provide homogeneous fluorescencebased 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 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.

### 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.

## Directions for use

### Kit components

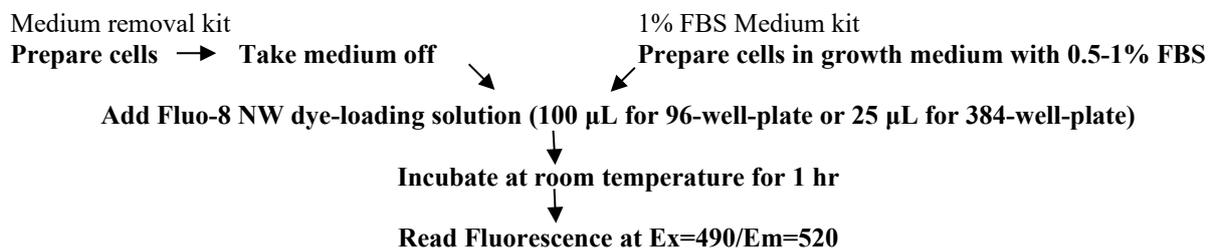
<u>Materials</u>	<u>10 plates</u>	<u>100 plates</u>
Component A: Fluo-8 NW	1 vial, lyophilized	10 vials, lyophilized
Component B: 10X Pluronic F127 Plus	10 bottles (1 ml/bottle)	10 bottles (10 ml/bottle)
Component C: HHBS (Hanks' with 20 mM HEPES)	1 bottle (100 ml)	Not included

### 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.

### Protocol 1- (for 1 plate)

#### Brief Summary



**Warning: No addition of probenecid is needed. Longer than 2 hours dye loading is not recommended.**

#### 1- Prepare cells

- 1.1 For adherent cells, plate cells overnight in growth medium 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/25 µL 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 For **Medium removal kit**, Make Fluo-8 NW stock solution by adding 10 µL (for Cat# **CJ256E**) or 100 µL (for Cat# **CJ2560** and # **CJ2561**) DMSO into component A (Fluo-8 NW), mixing them well.

*Note: 10 µL of reconstituted Fluo-8 NW is enough for 1 plate, unused 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.*

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For **1% FBS Medium kit**, 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 freezethaw cycles.*

### 2.3 Make 1X assay buffer

- a). For **10 plates kit**, make 1X assay buffer by adding **9 ml** component C (HHBS) into component B (10 X Pluronic F127 Plus, 1 ml), mix them well.
- b). For **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 For **Medium removal kit**, make Fluo-8 NW dye-loading solution for one cell plate by adding 10 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.

For **1% FBS Medium kit**, 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 For **Medium removal kit**, remove the growth medium from the cell plates

*Note1: It is important to remove the growth medium in order to minimize background fluorescence, and compound interference with serum or culture media.*

*Note2. Alternatively, one can grow the cells in growth medium with 0.5-to 1% FBS to avoid medium removal step, in this case, make 2X dye loading solution in HHBS buffer (We offer 2 separate no wash calcium assay kits (#CJ2550 and CJ2551) for people who uses 0.5-to 1% FBS in growth medium to avoid the medium removal step).*

For **1% FBS Medium kit**, proceed to step 3.2.

### 3.2 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) Fluo-8 NW dye-loading solution.

### 3.3 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.*

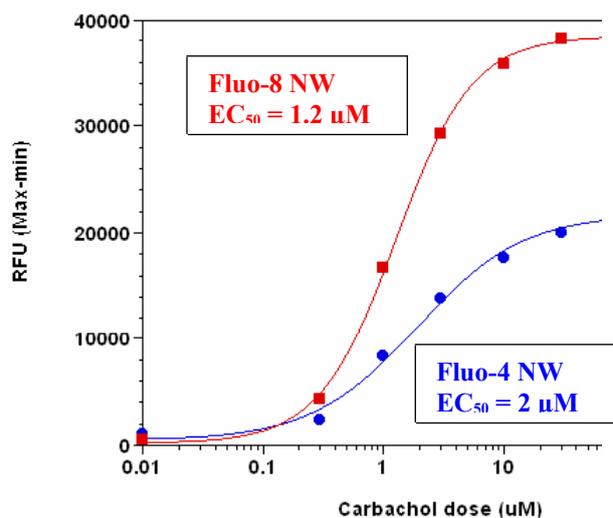
*Note 2: if the cells can be tolerated at room temperature for longer time, incubate the cell plate at room temperature for 1-2 hours. (longer than 2 hours is not recommended)*

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

### 3.5 Run the calcium flux assay by monitoring the fluorescence at Ex=490/Em=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 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 its signal test intensity around 7,000 to 10,000.*

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**Data Analysis**


**Fig1 : Carbachol Dose Response in HEK-293 cells measured with Fluo-8 NW Assay kit and Fluo-4 NW Assay Kit.** HEK-293 cells were seeded overnight in 40,000 cells per 100  $\mu\text{L}$  per well in a 96-well black wall/clear bottom plate. The growth medium was removed, and the cells were incubated with 100  $\mu\text{l}$  of the Fluo 8-NW calcium assay kit, or Fluo-4 NW kit (according to the manufacture's instructions) for 1 hour at room temperature. Carbachol (25 $\mu\text{l}$ /well) was added to achieve the final indicated concentrations. The  $\text{EC}_{50}$  of Fluo-8 NW is about 1.2  $\mu\text{M}$ .

**References**

- **Falk S. et al.**, Neurons in the preBötzing complex and VRG are located in proximity to arterioles in newborn mice. *Neuroscience Letters*. Volume 450, Issue 3, 229-234 (2009) [Abstract](#)
- **Ghoneum, M. et al.** *S. cerevisiae* induces apoptosis in human metastatic breast cancer cells by altering intracellular  $\text{Ca}^{2+}$  and the ratio of Bax and Bcl-2, *Int. J. of Oncology*, 33:533-539 (2008) [Article](#)
- **Ghoneum, M. et al.** An iron-based beverage, HydroFerrate fluid (MRN-100), alleviates oxidative stress in murine lymphocytes *in vitro*. *Nutrition Journal* 8:18-23 (2009) [Article](#)
- **Norez C. et al.**, A CF respiratory epithelial cell chronically treated by miglustat acquires a non-CF like phenotype, *Am. J. Respir. Cell Mol. Biol.*, 10.1165/rcmb.2008-0285OC (2009) [Abstract](#)

**Related products**

- Coelenterazine (native), [UP972333](#)
- Coelenterazine 400a, [UPBB8392](#)
- Coelenterazine H, [UPR3078B](#)
- Fluo-3 AM, [FP-78932C](#)
- Fluo-8 AM, [CP7502](#)
- Fluo-8H AM, [CP7531](#)
- Fluo-8L AM, [CP7551](#)
- Indo-1 AM, [FP-42775A](#)
- Rhod-2 AM, [FP-661583](#)
- Rhod-4 AM, [CQ6060](#)
- Fura-2 AM, [FP-42776C](#)
- Pluronic F-127, 20% solution, [FP-69806A](#)
- Probenecid, Cell culture tested, [FP-288652](#)
- Probenecid, water soluble, [FP-288653](#)
- Calcium calibration buffer kit, [FP-21527A](#)
- DMSO anhydrous, [JW7390](#)
- Black wall, clear bottom assay plate (96-well) [KT225](#), (384-well), [BA8170](#)

**Ordering information**

Catalog size quantities and prices may be found at <http://www.fluoprobes.com>  
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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