# Screen Quest<sup>TM</sup> Fluo-8 NW Calcium Assay Kit \*Medium Removal\*

*Ordering Information:* Product Number: #36308 (10 plates), #36309 (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<sup>™</sup> 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 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                           | #36308 (10 plates)       | #36309 (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              |

ABD Bioquest, Inc., 923 Thompson Place, Sunnyvale, CA 94085. Tel: 408-733-1055; Fax: 408-733-1304 Ordering: sales@abdbioquest.com; 800-990-8053 or 408-733-1055 Technical Support: support@abdbioquest.com; 408-733-1055

## 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 → Take medium off → Add Fluo-8 NW dye-loading solution (100 µL for 96well-plate or 25 µL for 384-well-plate) → Incubate at room temperature for 1 hr → 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 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 Fluo-8 NW dye-loading solution (see steps 2.4) 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 <u>Make Fluo-8 NW stock solution</u> by adding 100 µl DMSO into component A (Fluo-8 NW), mixing them well.

*Note:* 10  $\mu$ l of reconstituted Fluo-8 NW is enough for 1 plate, un-used reconstituted Fluo-8 NW can be aliquoted and stored at  $\leq -20^{\circ}$ C for more than one month if the tubes are sealed tightly, avoiding light and repeated freeze-thaw cycles.

- 2.3 <u>Make 1X assay buffer</u>

  a). For Cat# 36308 (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 Cat# 36309 (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 <u>Make Fluo-8 NW dye-loading solution for one cell plate</u> 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.

#### 3. Run Calcium Assay

- 3.1 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 (#36315 and 36316) for people who uses 0.5-to 1% FBS to avoid the medium removal step).
- 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.
- 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.

Data Analysis



Figure 1. Carbachol Dose Response in HEK-293 cells measured with Screen Quest<sup>TM</sup> Fluo-8 NW Assay kit and Fluo-4 NW Assay Kit. HEK-293 cells were seeded overnight in 40,000 cells per 100  $\mu$ L per well in a 96-well black wall/clear bottom costar plate. The growth medium was removed, and the cells were incubated with 100  $\mu$ l of the Screen Quest<sup>TM</sup> 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 $\mu$ l/well) was added by NOVOstar (BMG LabTech) to achieve the final indicated concentrations. The EC<sub>50</sub> of Fluo-8 NW is about 1.2 uM.

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.

ABD Bioquest, Inc., 923 Thompson Place, Sunnyvale, CA 94085. Tel: 408-733-1055; Fax: 408-733-1304 Ordering: sales@abdbioquest.com; 800-990-8053 or 408-733-1055 Technical Support: support@abdbioquest.com; 408-733-1055

## **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 <sup>™</sup> AM *Cell-permeable*  | 1 mg       |
| 21081 | Quest Fluo-8 <sup>™</sup> , AM *Cell-permeable*                                      | 5x50 μg    |
| 21082 | Ouest Fluo-8 <sup>TM</sup> , AM *Cell-permeable*                                     | 10x50 µg   |
| 21083 | Quest Fluo-8 <sup>TM</sup> , AM *Cell-permeable*                                     | 20x50 μg   |
| 21090 | Quest Fluo-8H <sup>™</sup> , AM *Cell-permeable*                                     | 1 mg       |
| 21091 | Ouest Fluo-8H <sup>™</sup> , AM *Cell-permeable*                                     | 10x50 µg   |
| 21096 | Quest Fluo-8L <sup>™</sup> , AM *Cell-permeable*                                     | 1 mg       |
| 21097 | Quest Fluo-8L <sup>™</sup> , AM *Cell-permeable*                                     | 10x50 μg   |
| 21120 | Quest Rhod-4 <sup>TM</sup> , AM  | 1 mg       |
| 21121 | Quest Rhod-4 <sup>TM</sup> , AM  | 5x50 ug    |
| 21122 | Quest Rhod-4 <sup>TM</sup> , AM  | 10x50 ug   |
| 21123 | Quest Rhod-4 <sup>TM</sup> , AM  | 20x50 μg   |
| 36301 | Screen Quest <sup>™</sup> 10X calcium assay buffer                                   | 10 Plates  |
| 36302 | Screen Quest <sup>™</sup> 10X calcium assay buffer                                   | 100 Plates |
| 36303 | Screen Quest <sup>™</sup> 10X calcium assay buffer with Phenol Red Plus <sup>™</sup> | 10 Plates  |
| 36305 | Screen Quest <sup>™</sup> Coelenterazine Calcium Assay Kit *10 Plates*               | 1 kit      |
| 36306 | Screen Quest <sup>™</sup> Coelenterazine Calcium Assay Kit *10X10 Plates*            | 1 kit      |
| 36307 | Screen Quest <sup>™</sup> Fluo-8 NW Calcium Assay Kit *Medium Removal*               | 1 Plate    |
| 36308 | Screen Quest <sup>™</sup> Fluo-8 NW Calcium Assay Kit *Medium Removal*               | 10 Plates  |
| 36309 | Screen Quest <sup>™</sup> Fluo-8 NW Calcium Assay Kit *Medium Removal*               | 100 Plates |
| 36314 | Screen Quest <sup>™</sup> Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium*         | 1 Plate    |
| 36315 | Screen Quest <sup>™</sup> Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium *        | 10 Plates  |
| 36316 | Screen Quest <sup>™</sup> Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium*         | 100 Plates |
| 36330 | Screen Quest <sup>™</sup> Rhod-4 NW Calcium Assay Kit *Medium Removal*               | 1 Plate    |
| 36331 | Screen Quest <sup>™</sup> Rhod-4 NW Calcium Assay Kit *Medium Removal*               | 10 Plates  |
| 36332 | Screen Quest <sup>™</sup> Rhod-4 NW Calcium Assay Kit *Medium Removal*               | 100 Plates |
| 36333 | Screen Quest <sup>™</sup> Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium*         | 1 Plate    |
| 36334 | Screen Quest <sup>™</sup> Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium *        | 10 Plates  |
| 36335 | Screen Quest <sup>™</sup> 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 <sup>™</sup> , sodium salt *0.1 M aqueous solution*                  | 10 mL      |
| 2457  | Trypan Red Plus <sup>™</sup> , sodium salt *0.1 M aqueous solution*                  | 100 mL     |
| 2455  | Trypan UltraBlue <sup>™</sup> , sodium salt  | 1 g        |