# Screen Quest™ Rhod-4 NW Calcium Assay Kit

**1% FBS Medium**

| *Ordering Information:* | *Storage Conditions:* | *Instrument Platform:* |
|---|---|---|
| Product Number: #36334(10 plates), #36335(100plates) | Keep in freezer and avoid 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™ Rhod-4 NW Calcium Assay Kit provides a homogeneous fluorescence-based assay for detecting the intracellular calcium mobilization. Cells expressing a GPCR of interest that signals through calcium are pre-loaded with our proprietary Rhod-4 NW which can cross cell membrane. Rhod-4 is the brightest red calcium indicator available for HTS screening. Once inside the cell, the lipophilic blocking groups of Quest Rhod-4™ are cleaved by non-specific cell esterase, 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 screening compounds, the receptor signals release of intracellular calcium, which greatly increase the fluorescence of Rhod-4. The characteristics of its long wavelength, high sensitivity, and >250 times fluorescence increases (when it forms complexes with calcium) make Quest Rhod-4™ an ideal indicator for measurement of cellular calcium. This Screen Quest™ Rhod-4 NW Calcium Assay Kit provides 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

| | |
|---|---|
| ***Longer Wavelengths:*** | Multiple excitations at 488, 514, 532 and 546 nm; Maximum emission at ~555 nm. |
| ***Larger Assay Window:*** | 2 fold brighter and 10 fold larger assay window than Rhod-2 AM. |
| ***Convenient and Robust:*** | Formulated to have minimal hands-on time. No wash required. |
| ***Versatile applications:*** | Compatible with many cell lines and receptors. |

## Kit Components

| **Components** | #36334 (10 plates) | #36335 (100 plates) |
|---|---|---|
| Component A: Rhod-4 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 |

## 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/Em = 488~545/555~590 (optimal Ex/Em = 540/590 nm).
- HHBS (1X Hank's with 20 mM Hepes buffer, pH 7.0).
- 100% DMSO.

## Assay Protocol for One 96-well Plate

### Brief Summary

**Prepare cells in growth medium with 0.5-1% FBS → Add Rhod-4 NW dye-loading solution (100 µL for 96-well plate or 25 µL for 384-well plate) → Incubate at room temperature for 1 hr → Read Fluorescence at Ex/Em = 540/590 nm**

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

1.2 For non-adherent cells, centrifuge the cells from the culture medium and then suspend the cell pellets in Rhod-4 NW dye-loading solution (see steps 2.4) at 125,000 to 250,000 cells/well/100 µL 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 Rhod-4 NW dye-loading solution:**

2.1 Thaw 1 vial of Component A (Rhod-4 NW), 1 bottle of Component B (10X Pluronic F127 Plus) and Component C (HHBS) at room temperature before use.

2.2 Make Rhod-4 NW stock solution by adding 200 µL DMSO into Component A (Rhod-4 NW), mixing them well.
*Note: 20 µLof reconstituted Rhod-4 NW is enough for 1 plate, unused reconstituted Rhod-4 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.*

2.3 Make 1X assay buffer
a). For **Cat# 36334 (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# 36335 (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 of 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 Make Rhod-4 NW dye loading solution for one cell plate by adding 20 µL of DMSO reconstituted Rhod-4 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 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) Fluo-8 NW dye-loading solution (from step 2.4) into the cell plate.
*Note: Alternatively, one can grow the cells in growth medium with 5 to 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 no wash calcium assay kits (#36331 and 36332) for people who prefer the medium removal step).*

3.2 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.*

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

3.4 Run the calcium flux assay by monitoring the fluorescence at Ex/Em = 540/590 nm.

## Data Analysis

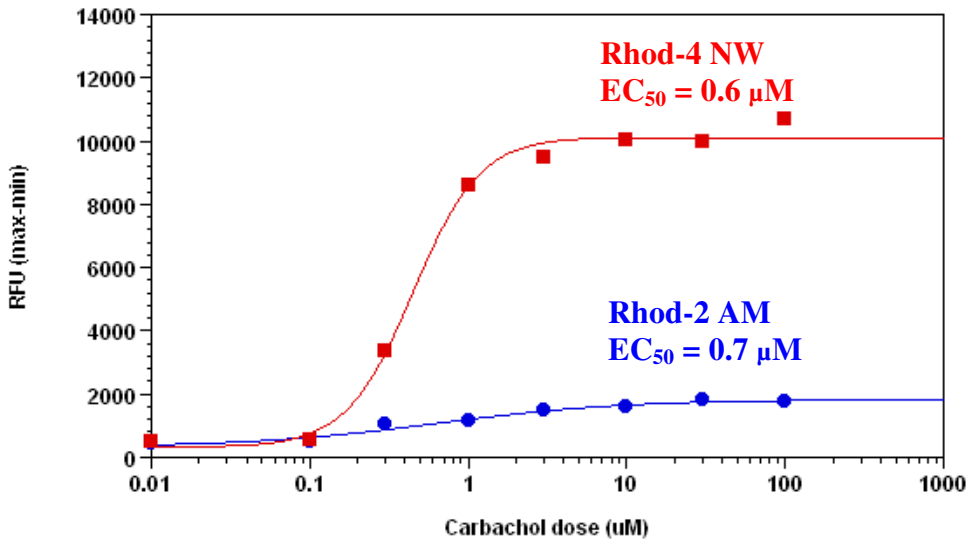



**Figure 1. Carbachol Dose Response in HEK-293 cells measured with Screen Quest™ Rhod-4 NW Assay kit and Rhod-2 AM.** HEK-293 cells were seeded overnight in 40,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. The cells were incubated with 100 µL of the Screen Quest™ Rhod-4 NW calcium assay kit, or 5 µM of Rhod-2 AM 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 Rhod-4 NW is about 0.6 µM.

**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.**