Screen Quest™ Rhod-4 NW Calcium Assay Kit

Medium Removal

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36331 (10 plates), 36332 (100 plates)	Keep in freezer Protect from light	FLIPR, FDSS, NOVOStar, FlexStation, ViewLux, IN Cell Analyzer, ArrayScan

Introduction

Cells expressing a GPCR of interest that signals through calcium are pre-loaded with our proprietary Rhod-4 NW which can cross cell membrane. Quest 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 esterases, resulting in a negatively charged fluorescent dye which stays inside the cells. Its fluorescence is greatly enhanced upon binding to calcium. When cells stimulated with screening compounds, the receptor signals release of intracellular calcium, which significantly increase the fluorescence of Quest Rhod-4™. The characteristics of its long wavelength, high sensitivity, and >250 times fluorescence increases make Quest Rhod-4™ an ideal indicator for the measurement of cellular calcium. These Screen Quest™ Rhod-4 NW Calcium Assay Kits provide an optimized assay method for monitoring the 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. Compared to Fluo-3, Fluo-4 and Quest Fluo-8™, Quest Rhod-4™ is more photostable, making its fluorescence imaging more robust.

Kit Key Features

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

Kit Components

Components	**Amount**	
	Cat. # 36331 (10 plates)	**Cat. # 36332 (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)

- A 96 or 384-well microplate: A tissue culture microplate with black wall and clear bottom.
- A fluorescence microplate reader with a filter set of Ex = 488 to 545 nm and Em = 555 to 590 nm (optimal Ex/Em = 540/590 nm).
- HHBS (1X Hank's with 20 mM Hepes Buffer, pH 7.0).
- 100% DMSO.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

Assay Protocol for One 96-well Plate

Brief Summary

Prepare cells → Remove the growth medium→ Add Rhod-4 NW dye-loading solution (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Incubate at room temperature for 1 hour → Monitor fluorescence intensity at Ex/Em = 540/590 nm

Warning: Do not add additional probenecid.

1. Prepare Cells:

1.1 For adherent cells: Plate cells overnight in the 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 Rhod-4 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/25 µL for a 384-well poly-D lysine plate. Centrifuge the plates 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 Rhod-4 NW dye-loading solution:

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

2.2 Make Rhod-4 NW stock solution: Add 100 µL of DMSO into the vial of Rhod-4 NW (Component A), and mix them well.
Note: 10 µL of Rhod-4 NW stock solution is enough for one plate. Unused Rhod-4 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. # 36331 (10 plates kit)**: make 1X assay buffer adding **9 mL** of HHBS (Component C) into the bottle of 10X Pluronic® F127 Plus, (1 mL, Component B), and mix them well.

b). For **Cat. # 36332 (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 Rhod-4 NW dye-loading solution for one cell plate by adding 10 µL of DMSO reconstituted Rhod-4 NW (from Step 2.2) into 10 mL of 1X assay buffer (from Step 2.3), and 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 plate.
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, grow the cells in growth medium with 0.5-1% 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. # 36334 and Cat. # 36335) for those who use 0.5-1% 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 Rhod-4 NW dye-loading solution (from Step 2.4) into the cell plates (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.

3.4 Prepare the compound plate with HHBS or the desired buffer.

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

Data Analysis

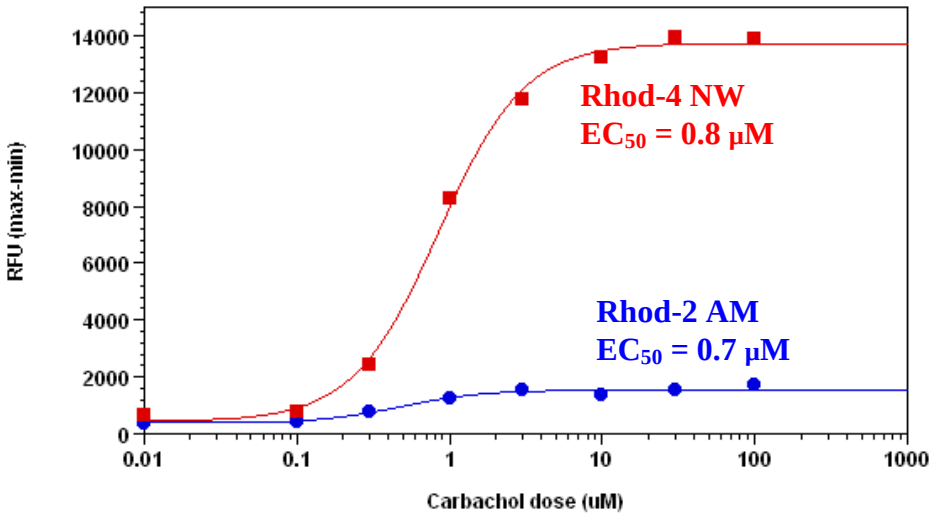

Figure 1. Carbachol Dose Response was measured in HEK-293 cells with Screen Quest™ Rhod-4 NW Assay kit and Rhod-2 AM. HEK-293 cells were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The growth medium was removed, and the cells were incubated with 100 µL of dye loading solution using the Screen Quest™ Rhod-4 NW calcium assay kit, or 100 µL of Rhod-2 AM solution (5 µM) for 1 hour at room temperature. Carbachol (25 µL/well) was added by NOVOstar (BMG Labtech) to achieve the final indicated concentrations. The EC_{50} of Cabachol by using Rhod-4 NW is about 0.8 µM.