Screen Quest™ Rhod-4 NW Calcium Assay Kits

1% FBS Growth Medium

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36334(10 plates), 36335(100 plates)	Keep in freezer Protect from light	FLIPR, FDSS, NOVOStar, FlexStation, ViewLux, IN Cell Analyzer, ArrayScan

Introduction

Screen Quest™ Rhod-4 NW Calcium Assay Kits provide 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 esterases, resulting in a negatively charged fluorescent dye that stays inside the cells. Its fluorescence is greatly enhanced upon binding to calcium. When cells stimulated with screening compounds, the receptor signals the release of intracellular calcium, which greatly increase the fluorescence of Rhod-4. 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 kits come with all the essential components with an optimized protocol to use with FLIPR® or FDSS™ or an equivalent instrument.

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 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. # 36334 (10 plates)	**Cat. # 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 microplates: Tissue culture microplate with black wall and clear bottom.
- A fluorescence microplate reader 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.

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 in growth medium with 0.5-1% FBS → 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 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 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 Rhod-4 NW dye-loading solution:

2.1 Thaw all the kit components at room temperature before use.

2.2 Make Rhod-4 NW stock solution: Add 200 µL of DMSO into the vial of Rhod-4 NW (Component A), and mix them well.
Note: 20 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. # 36334 (10 plates kit)**, make 1X assay buffer by 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. # 36335 (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 un-used 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: Add 20 µL of Rhod-4 NW 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 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 plate.
Note: Alternatively, 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 (Cat. # 36331 and 36332) for people who prefer to keep the medium removal step].

3.2 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.

3.3 Prepare the compound plate with HHBS or your desired buffer.

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

Data Analysis

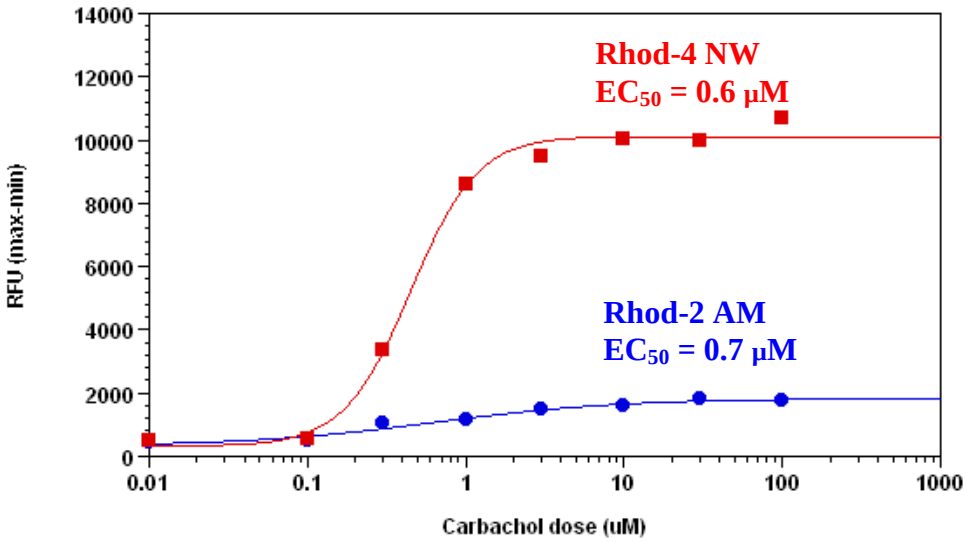

Figure 1. Carbachol Dose Response was measured in HEK-293 cells with Screen Quest™ Rhod-4 NW Assay kit and Rhod-2 AM**.** HEK-293 cells were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of dye-loading solution using 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 (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.