

Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal*

Ordering Information:

Product Number: #36331 (10 plates), #36332 (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™ 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 (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

- 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

Materials	#36331 (10 plates)	#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

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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 = 488 to 545 nm and Em= 555 to 590 (optimal Ex = 530 nm/Em = 570 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 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=530/Em=570

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 Rhod-4 NW dye-loading solution (for 1 plate)

- 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 100 µl DMSO into component A (Rhod-4 NW), mixing them well.
Note: 10 µl of 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# 36331 (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# 36332 (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 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), 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 (#36334 and 36335) for people who uses 0.5-to 1% FBS to avoid the medium removal step).

3.2 Add 100 μ L/well (96-well plate) or 25 μ L/well (384-well plate) Rhod-4 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=530/Em=570 nm.

Data Analysis

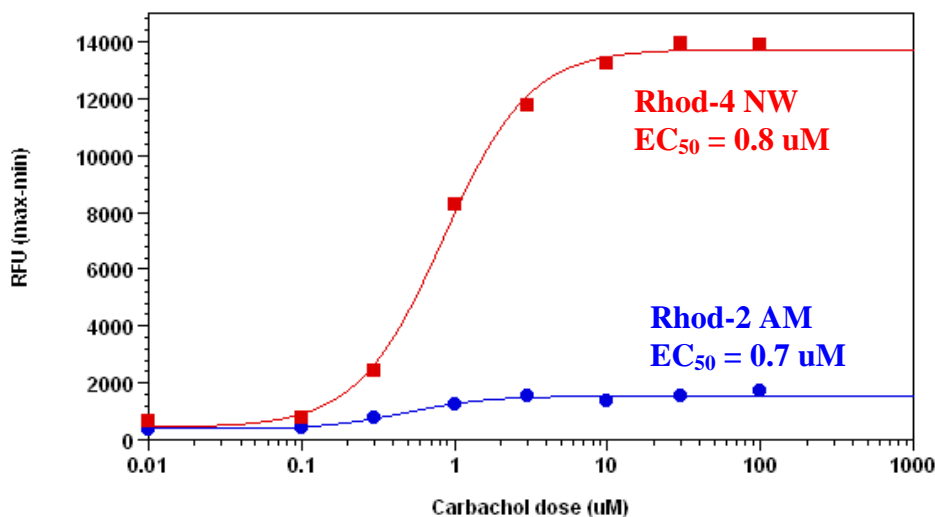


Figure 1. **Carbachol Dose Response in HEK-293 cells measured with Screen Quest™ Rhod-4 NW Assay kit and Fluo-4 NW Assay Kit.** HEK-293 cells were seeded overnight in 40,000 cells per 100 μ L per well in a 96-well black wall/clear bottom costar plate. The growth medium was removed, and the cells were incubated with 100 μ l of the Screen Quest™ Rhod-4 NW calcium assay kit, or 5 uM of Rhod-2 AM for 1 hour at room temperature. Carbachol (25 μ l/well) was added by NOVOstar (BMG LabTech) to achieve the final indicated concentrations. The EC₅₀ of Rhod-4 NW is about 0.8 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.

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

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