Screen Quest™ Rhod-4 No Wash Calcium Assay Kit

Catalog number: 36334, 36335, 36333
Unit size: 10 Plates, 100 Plates, 1 Plate

Component	Storage	Amount		
		Cat No. 36334	Cat No. 36335	Cat No. 36333
Component A: Rhod-4 NW	Freeze (<-15 °C), Minimize light exposure	1 vial, lyophilized	10 vials, lyophilized	1 vial, lyophilized
Component B: 10X Pluronic® F127 Plus	Freeze (<-15 °C), Minimize light exposure	10 bottles (1 mL/bottle)	10 bottles (10 mL/bottle)	1 bottle (1 mL)
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	Freeze (<-15 °C), Minimize light exposure	1 bottle (100 mL)	Not included	1 bottle (9 mL)

OVERVIEW

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 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 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.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium with 1-5% FBS
2. Add Rhod-4 NW dye-loading solution (100 µL/well for 96-well plate or 25 µL/well for 384-well plate)
3. Incubate at room temperature for 1 hour
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm

Important Thaw all the kit components at room temperature before starting the experiment. Do not add additional probenecid.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode/Programmable liquid handling

Other Instruments: FLIPR, FDSS, NOVOStar, FlexStation, ViewLux, IN Cell Analyzer, ArrayScan

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Rhod-4 NW stock solution:
Add 200 µL of DMSO into the vial of Rhod-4 NW (Component A) and mix well. Protect from light.

Note 20 µL of Rhod-4 NW stock solution is enough for one plate.

Note 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. Assay Buffer (1X):
a) For **Cat. # 36333 (1 plate kit) and # 36334 (10 plates kit)** , make 1X assay buffer by adding 9 mL of HHBS (Component C) into 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 90 mL of HHBS (Not included) into 10X Pluronic® F127 Plus (10 mL, Component B), 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.

PREPARATION OF WORKING SOLUTION

Rhod-4 NW dye-loading solution:
Add 20 µL of Rhod-4 NW stock solution into 10 mL of 1X assay buffer, and mix them well.

Note This working solution is stable for at least 2 hours at room temperature.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Rhod-4 NW dye-loading solution into the cell plate.
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 If the assay requires 37 °C, perform the experiment immediately without further room temperature incubation.

   Note If the cells can function well at room temperature for longer time, incubate the cell plate at room temperature for 1-2 hours.
3. Prepare and add the compound plate with HHBS or your desired buffer.
4. Run the calcium flux assay by monitoring the fluorescence intensity at Ex/Em = 540/590 nm.

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EXAMPLE DATA ANALYSIS AND FIGURES

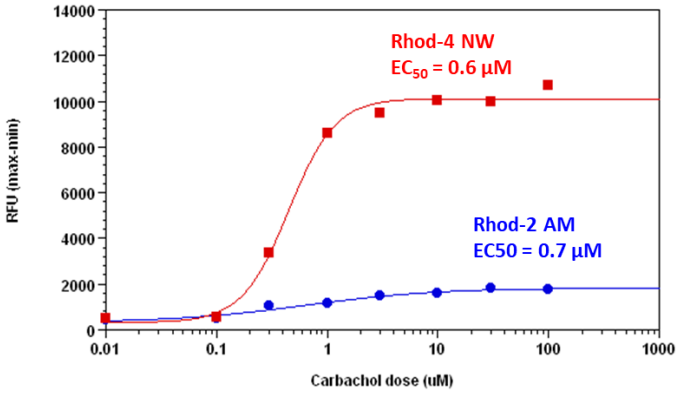

Figure 1. Carbachol Dose Response was measured in HEK-293 cells with Screen Quest™ Rhod-4 NW Assay kit and Rhod-2 AM**.** HEK-293 cells were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of dye-loading solution using 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 Carbachol using Rhod-4 NW is about 0.6 µM.

DISCLAIMER