abcam

Product datasheet

Rhod-4 Calcium Assay Kit - No Wash ab112157

4 References 1 Image

Overview

Product name Rhod-4 Calcium Assay Kit - No Wash

Detection method Fluorescent

Sample type Adherent cells, Suspension cells

Assay type Cell-based
Assay time 1h 00m

Species reactivity Reacts with: Mammals, Other species

Product overview Abcam Rhod-4 No Wash Calcium Assay Kit (ab112157) is a fluorescence-based assay for

Abcam Rhod-4 No Wash Calcium Assay Kit (ab112157) is a fluorescence-based assay for detecting intracellular calcium mobilization. Cells expressing a GPCR of interest that signals through calcium are pre-loaded with Rhod-4 which can cross the cell membrane. Once inside the cell, the lipophilic blocking groups of Rhod-4 are cleaved by an esterase, resulting in a negatively charged fluorescent dye that stays inside the cell. Its fluorescence is greatly enhanced upon binding to calcium. When cells are stimulated with agonists, the receptor signals the release of intracellular calcium, which significantly increases the fluorescence of Rhod-4. The characteristics of its long wavelength, high sensitivity, and >250 times fluorescence enhancement make Rhod-4 the brightest red calcium indicator available in the marker, and it is an ideal tool for the measurement of cellular calcium through HTS screening. Compared to Fluo-8, Rhod-4 is more photostable, making its fluorescence imaging more robust.

ab112157 provides an optimized assay method for monitoring the G-protein-coupled receptors and calcium channels using HTS instrumentation. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format.

Visit our FAQs page for tips and troubleshooting.

Notes This product is intended to be used for monitoring calcium fluctuations in vivo in live cells using the

following HTS imaging plate readers: FLIPR™, FDSS, BMG NOVOstar™, FLexStation, ViewLux,

IN Cell Analyzer or Arrayscan.

If you would like to quantify calcium concentration $in\ vitro$ using cell extracts, we recommend using

Calcium Detection Kit (Colorimetric) (ab102505) or Calcium Quantification Assay Kit

(ab112115), as they provide stock standards for comparison.

Platform Microplate reader

Proportios

1

Storage instructions

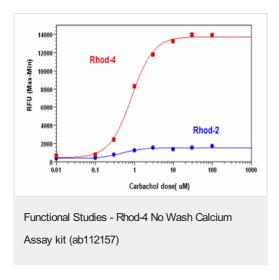
Store at -20°C. Please refer to protocols.

Components	1 kit
10X Pluronic® F127 Plus	1 x 1ml
HHBS	1 x 9ml
Rhod-4	1 vial

Relevance

Calcium is essential for all living organisms, where Ca²⁺ sequestration and release into and out of the cytoplasm functions as a signal for many cellular processes. 99% of calcium is found in bones and teeth with the remaining 1% found in the blood and soft tissue. Serum calcium levels are tightly controlled (8.4-11.4 mg/dL) and any variation outside this range can have serious effects. Calcium plays a role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and hormone secretion. Calcium ion channels control the migration of calcium ions across cell membranes, permitting the activation and inhibition of a wide variety of enzymes. Causes of low calcium levels include chronic kidney failure, vitamin D deficiency, and low blood magnesium levels that can occur in severe alcoholism.

Images



Carbachol Dose Response was measured in HEK293 cells with ab112157 and Rhod-2. HEK293 cells were seeded overnight at 40,000 cells/100 µL/well in a black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of dye-loading solution using ab112157, or 100 µL of Rhod-2 solution (5 µM) for 1 hour at room temperature. Carbachol (25 µL/well) was added to achieve the final indicated concentrations. The EC50 of Rhod-4 is about 0.6 µM, and that for Rhod-2 is about 0.7 µM.

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