A New Robust No-Wash FLIPR Calcium Assay Kit for Screening GPCR and Calcium Channel Targets

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Introduction

Calcium flux assays are preferred methods in drug discovery for screening G protein coupled receptors (GPCRs). Screen Quest™ Fluo-8 No Wash Calcium Assay Kits provide homogeneous fluorescence-based assays for detecting intracellular calcium mobilization across a broad spectrum of biological targets. The characteristics of its long wavelength, high sensitivity and ≤200 times fluorescence enhancement (when it forms a complex with calcium) make Fluo-8 NW an ideal indicator for measurement of cellular calcium.

The signal intensity and well-to-well uniformity of Fluo-8 NW is compared with FLUO-3 AM, Fluo-4 NW and FLUO-4 AM wash procedures. The Screen Quest™ Fluo-8 NW yields the brightest signal, more than 2 fold brighter than Fluo-4 AM, and 4 times brighter than Fluo-3 AM. The Screen Quest™ Fluo-8 NW Calcium Assay Kits provide an optimized assay method for monitoring GPCRs and calcium channels. Unlike Fluo-3 AM and Fluo-4 AM, both of which require 37°C for optimal cell loading, the Screen Quest™ Fluo-8 NW requires a less temperature-dependent cell loading property, giving similar results either at room temperature or 37°C. This characteristic makes the Screen Quest™ Fluo-8 NW Calcium Assay Kits more robust for HTS applications.

Material and Methods

1. CHO-M1 or HEK cells were plated at 96-well (for NOVOstar) or 384-well black wall/clear bottom costar plate (for FLIPR) at 37°C incubator for overnight.

2. Take 2.5 µL of Fluo-3 AM, Fluo-4 AM or Fluo-8 NW at room temperature for 1 hr at (or at 37°C for 30 min, then at room temperature for 30 min).

For wash experiments: wash cells with HHBS buffer twice, then replace with HHBS buffer.

4. For No wash experiments: run the experiments directly.

5. Run calcium efflux experiments on NOVOstar or FLIPR

Plating cells for overnight

Aspirate growth medium (Skip this if cells in 0.5-1% FBS)

Dye loading for 1 hr at RT or 37°C

Run calcium assay at Ex 485-490nm/Em 520-530nm

Results

Figure 1. Cells were seeded overnight in 40,000 cells per 100 µl per well in a 96-well black wall/clear bottom costar plate. The cells were incubated with 100 µl of Screen Quest™ Fluo-8 NW Calcium Assay kit, or Fluo-4 NW kit (based on manufacturer’s protocol) for 1 hour at room temperature. Carbachol (100 µM) was added by NOVOstar (BMG Labtech) to achieve the final indicated concentration. The EC 50 was about 2 µM which is similar as reported.

Figure 5. Carbachol-Dose Response in HEK-293. HEK-293 cells were seeded overnight in 10,000 cells per 25 µl per well in a 384-well black wall/clear bottom costar plate. The growth medium was removed, and half of the plate was incubated with 25 µl of the Screen Quest™ Fluo-8 NW Calcium Assay kit, and the other half of the plate were incubated with Fluo-4 NW kit (based on manufacturer’s protocol) for 1 hour at room temperature. Carbachol (12.5 µM) was added by FLIPR to achieve the final indicated concentration. The EC 50 was about 4 µM which is similar as reported.

Figure 6. Comparisons of Screen Quest™ Fluo-8 NW Calcium Assay Kit, Fluo-4 NW on Concanavalin A induced Ca entry (competitive calcium entry (CaC1)) into Jurkat cells. Jurkat cells were suspened at 6x10^5 cells per ml in calcium-free HHBS buffer, cells were incubated with equal volume of Fluo-8 NW or Fluo-4 NW in calcium-free HHBS buffer at 2X10^5 cells/ml (100 µl) at a 96-well black wall/clear bottom costar plate for 1 hr at 37°C, 1% CO2 incubator. At the end of the 10 min incubation, the channel-opener Concanavalin A at 1 ug/ml was added to Fluo-8 NW and Fluo-4 NW. The signal intensity and well-to-well uniformity of Fluo-8 NW is compared with FLUO-3 AM, Fluo-4 NW and FLUO-4 AM wash procedures, the Screen Quest™ Fluo-8 NW yields the brightest signal, more than 2 fold brighter than Fluo-4 AM, and 4 times brighter than Fluo-3 AM. The Screen Quest™ Fluo-8 NW Calcium Assay Kits provide an optimized assay method for monitoring G-protein-coupled receptors (GPCRs) and calcium channels. Unlike Fluo-3 AM and Fluo-4 AM, both of which require 37°C for optimal cell loading, the Screen Quest™ Fluo-8 NW requires a less temperature-dependent cell loading property, giving similar results either at room temperature or 37°C. This characteristic makes the Screen Quest™ Fluo-8 NW Calcium Assay Kits more robust for HTS applications.

Summary

The Screen Quest™ Fluo-8 NW Calcium Assay kit has been optimized for broad range of instruments to give maximum performance with GPCR and calcium channel targets.

- Brighter: Enable calcium assays with demanding cell lines and receptors;
- 1536 Well-friendly: best Ca²⁺ indicator for 1536-well Ca²⁺ assays;
- Less temperature-Sensitive: 37 °C & RT loadings generate similar results;
- Larger assay window: Capable of assaying the challenging cell lines and receptors.

Conclusions

The Screen Quest™ Fluo-8 NW Calcium Assay kit provides an optimized assay method for monitoring G-protein-coupled receptors (GPCRs) and calcium channels. Its ability to generate very large signal from the weak and robust assay systems enables researchers to have multiple assay chemistries for different receptor and calcium channel targets.