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

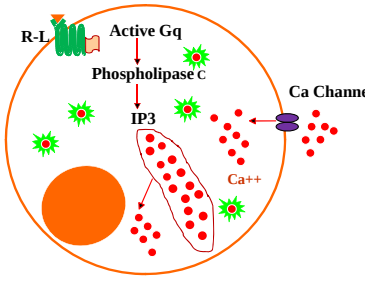
Jinfang Liao*, Jianjun He, Chunmei Wei, Wenping Chang and Zhenjun Diwu
ABD Bioquest, Inc., 923 Thompson Place, Sunnyvale, CA 94085

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 provides 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 Flu-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.

Screen Quest™ Fluo-8 Calcium Assay



[Ca^{2+}] Increase via Gq or calcium channel is measured by Quest Fluo-8™ fluorescence enhancement.

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 growth medium off (if cells were plating in 0.5-1% FBS, skip this step), incubate the cells with Fluo-3 AM, Fluo-4 AM or Fluo-8 NW at room temperature for 1-2 hr (or at 37°C for 30 min, then at room temperature for 30 min).
3. 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

Figure1. U2OS cells were seeded overnight at 40,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. The growth medium was removed, and the cells were incubated with 100 µl of 4 µM Fluo-3 AM, Fluo-4 AM or Quest Fluo-8™ AM in HHBS at 37 °C for 1 hour. The cells were washed twice with 200 µl HHBS, then imaged with a fluorescence microscope (Olympus IX71) using FITC channel.

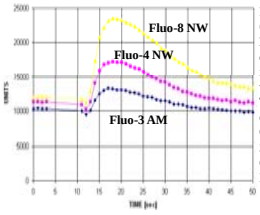


Figure 2. **Comparisons of Screen Quest™ Fluo-8 NW Calcium Assay Kit, Fluo-4 NW, and Fluo-3 AM in HEK-293 cells.** HEK-293 cells were seed overnight in 40,000 cells per 100 µl per well in a 96-well black wall/clear bottom costar plate. The growth medium was removed, and the cells were incubated with 100 µl of Screen Quest™ Fluo-8 NW, Fluo-4 NW, or Fluo-3 AM, for 1 hr at 37 °C. 30 µM of Carbachol (50µl/well) was added by NOVOstar (BMG Labtech).

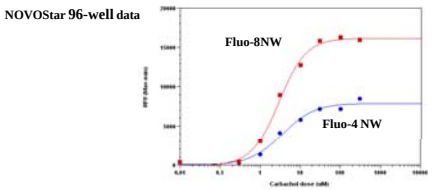


Figure 3. **Carbachol Dose Response in HEK-293.** HEK-293 cells were seed overnight in 40,000 cells per 25 µl per well in growth medium with 1%FBS in a 96-well black wall/clear bottom costar plate. The cells were incubated with 100 µl of the Screen Quest™ Fluo-8 NW Calcium Assay kit, or Fluo-4 NW kit (based on manufacture's protocol) for 1hour at room temperature. Carbachol (50µl/well) was added by NOVOstar (BMG labtech) to achieve the final indicated concentration. The EC 50 is about 2 µM which is similar as reported.

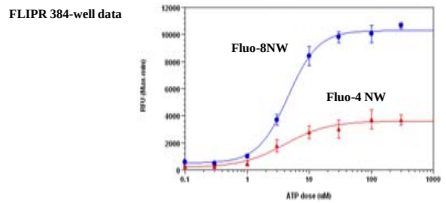


Figure 4. **ATP Dose Response in HEK-293.** HEK-293 cells were seeded overnight in 10,000 cells per 25 µl per well in a 384-well black wall/clear bottom costar plate. The growth medium was removed and the half of the plate was incubated with 25 µl of the Fluo-8 NW Calcium assay kit, and the other half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

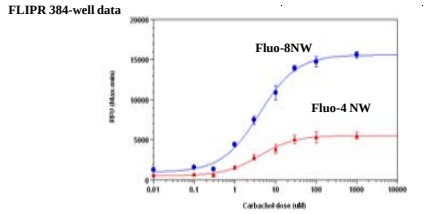


Figure 5. **Carbachol Dose Response in HEK-293.** HEK-293 cells were seeded overnight in 10,000 cells per 25 µl per well in a 384-well black wall/clear bottom costar plate. The growth medium was removed and the half of the plate was incubated with 25 µl of the Screen Quest™ Fluo-8 NW Calcium Assay kit,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

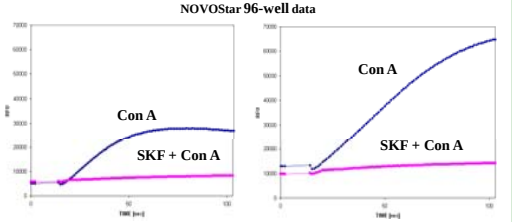


Figure 6. **Comparisons of Screen Quest™ Fluo-8 NW Calcium Assay Kit, Fluo-4 NW on Concanavalin A induced Ca entry** (capacitative calcium entry (CCE)) **into JurKat cells.** Jurkat cells were suspended at 4X10^6 cells per ml in calcium-free HHBS buffer, cells were incubated with equal volume of Fluo-8 NW or Fluo-4 NW in calcium-free HHBS buffer at 2X10^5 cells/well/100 µL at a 96-well black wall/clear bottom costar plate for 1 hr at 37 °C, 5% CO_2 incubator. At the end of the 10 min incubation, the channel opener Concanavalin A at 1 ug/ml with or without the channel inhibitor SKF96365 at 30 µM were added into the cells. 25 µL/well of HBSS with additional 24 mM (5X) Calcium (so final in well concentration of calcium is 5 mM) was added by NOVOstar (BMG Labtech).

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 Ca^{2+} indicator for 1536-well Ca^{2+} 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.

ABD Bioquest
Advancing Biological Detections through Innovations

For reprint please visit Booth 725