Evaluation of FLIPR Calcium Assays for Screening GPCR and Calcium Channel Targets
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Introduction

Quest Fluo-8™ AM, Fluo-4 Direct™ and Calcium-4™ have been compared and evaluated for several GPCR and ion channel targets. Quest Fluo-8 AM, Fluo-4 and Calcium 4 share the same assay principle. All the three fluorogenic calcium indicators are in the form of non-fluorescent AM esters. Once inside cells, the lipophilic AM blocking groups are cleaved by non-specific cellular esterases, resulting in negatively charged fluorescent dyes that stay inside cells, and their fluorescence intensities are greatly enhanced upon binding to calcium. When cells are stimulated with screening compounds, the receptor signals release of intracellular calcium, which greatly increases their fluorescence. Three GPCRs (Masurcanin M1 receptor, purergic P2Y receptor, and chemokine CCR2b receptor) and one calcium channel capacitative calcium entry (CCE) were assayed. In comparison with Fluo-4 Direct and Calcium 4, Quest Fluo-8 yields the brightest signal and largest assay window. Quest Fluo-8 AM has a lower temperature-dependent cell loading property, giving more consistent results either at room temperature or 37°C compared to Fluo-4 Direct or Calcium 4. This characteristic makes Quest Fluo-8 AM much more robust for HTS applications.

Material and Methods

1. CHO-M1, HEK or Jurkat cells were plated at 96-well black wall/clear bottom Costar plate at 37℃ incubator for overnight.
2. The cells were incubated with Fluo-4 Direct, Calcium 4 or Quest Fluo-8™ AM.
3. Run calcium efflux experiments on FlexStation.

**Plating cells for overnight**

**Dye loading for 1 hr at RT or 37℃**

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

Results

Figure 1. U2OS cells were seeded overnight at 30,000 cells per 100 µL per well in a 96-well black wall/clear bottom Costar plate. The cells were incubated with 100 µl of Fluo-4 Direct, Calcium 4, or Quest Fluo-8 AM at room temperature for 1 h. The cells were imaged with a fluorescence microscope (Olympus IX71) using FITC channel.

Figure 2. ATP Dose Response in CHO-M1 cells. CHO-K1 cells were seeded overnight in 50,000 cells per 100 µl per well in a 96-well black wall/clear bottom costar plate. The cells were incubated with 100 µl of Fluo-4 Direct, Calcium 4 or Quest Fluo-8 AM at 37°C, 5% CO2, incubator, and then another 30 min at RT. ATR (50 µl/well) was added by Flexstation to achieve the final indicated concentrations. The IC50 of ATP is about 10 µM which is similar as reported.

**Comparisons of Screen Quest Fluo-8 AM, Fluo-4 Direct, and Calcium 4 on Concanavalin A induced calcium entry (capacitative calcium entry (CCE)) into Jurkat cells. Jurkat cells were suspended at 2X10⁶ cells/ml in calcium-free HBSS buffer, cells were incubated with equal volume of Fluo-4 Direct, Calcium-4 and Quest Fluo-8 AM in HBBS buffer at 37 oC, final in well concentration is 5 µM calcium (so final in well concentration of calcium is 5 nM) was added by Flexstation**

Summary

Quest Fluo-8 AM provides an optimized assay method for monitoring GPCRs and calcium channels. Its ability to generate strong calcium signal from the weak receptors and difficult cell lines enables researchers to have multiple assay chemistries for different GPCR receptor and calcium channel targets. Quest Fluo-8 technology has the following benefits and features:

- **Brighter**
- **Enable Ca²⁺ assays with weak cell lines & receptors**
- **1536 Well-Friendly**
- **The best Ca²⁺ indicator for 1536-well Ca²⁺ assays**
- **Large Assay Window**
- **Large Z’ factor for challenging cell lines & receptors.**
- **Robust & Less Temperature-Sensitive**
- **37 °C & RT loadings generate similar results**