

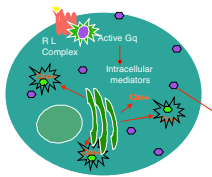
FLIPR® Calcium 3 Assay Kit: a new no-wash calcium flux assay for diversified receptor targets

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abstract

Cell-based calcium flux assays are considered one of the most important screening techniques used in pharmaceutical drug discovery today. Due to variability in receptor expression, host cell characteristics and assay dynamics, receptor targets often need to be matched with specific calcium flux chemistries to achieve acceptable performance. We report here, on the FLIPR Calcium 3 Assay Kit, a new "universal" fluorescence-based method for detecting changes in intracellular calcium concentration across a broad spectrum of biological targets. The Calcium 3 assay Kit yields very high fluorescence emission intensity whereby strong signals are attained even in problematic targets that may previously have yielded weak signal intensity or no peak at all. The simple and reliable "mix-and-read" assay format substantially reduces, or eliminates entirely, the cell detachment and diminished response often associated with incubate-and-read wash procedures. Data is presented that compares signal intensity and well-to-well uniformity observed with the Calcium 3 Assay Kit and standard FLUO-3 AM and FLUO-4 AM dye incubation and wash procedures, as well as previous mix-and-read kits available from Molecular Devices.

GPCR : Intracellular Mediators Calcium Pathway



Increase in cytosolic calcium can be detected by fluorescence measurement using calcium-sensitive dye indicators

materials and methods

Cell lines: CHO-M1 cells, CHO-CCR5-Gq5 cells, CHO-NCR-Gq5 cells and CHO-CCR2B-Ga16 cells (transient-transfection)

The fluorescence relative intensities were measured at 1 sec intervals by using FLIPR (Excitation at 488 nm, and 515 nm emission filter 1), or FlexStation™ (excitation at 485 nm and emission at 525 nm) (Molecular Devices Corporation).

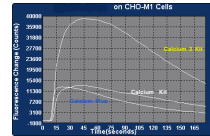
Data analysis: Graphs were generated from the peak fluorescence values obtained minus the minimum fluorescence. EC50 and IC50 values were estimated by using 4-parameter curve on Softmax Pro (Molecular Devices Corporation).

Procedures for Calcium 3 Kit vs. Fluo3 and Fluo4 wash

	Calcium 3	Fluo 3 or Fluo 4 Wash	
1. Cells plated at 100 ul/well (96 well) or 25 ul/well (384 well)	Same		or
2. Reagent added at 100 ul/well (96 well) or 25 ul/well (384 well), incubate 1 hr.	Different-- Aspirate the medium prior to dye loading		
3. Assay on FLIPR or FlexStation	Different-- Wash 3 times with HBSS before FLIPR or FlexStation assay		or 25 ul

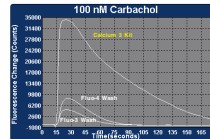
results

Comparison of the Calcium 3 Assay Kit with the Calcium Assay Kit and Calcium Plus Assay Kit on FLIPR



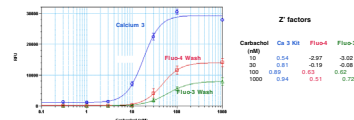
CHO-M1 cells were assayed overnight in 100 uL (50,000 cells) on a Costar black wellclear bottom plate. The cells were incubated with 100 uL of either the Calcium 3 Assay Kit, Calcium Plus Assay Kit, or the Calcium Assay Kit for 1 h at 37°C. The addition consisted of 50 uL (either at 100 nM) (n=3/group).

Comparison of the Calcium 3 Assay Kit with Fluo-4 wash and Fluo-3 wash on FLIPR



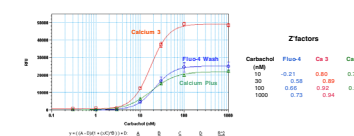
CHO-M1 cells were assayed overnight in 100 uL (50,000 cells) on a Costar black wellclear bottom plate. The cells were incubated with 100 uL of either the Calcium 3 Assay Kit, or the medium replaced with either 100 uL of Fluo-4 AM or Fluo-4 AM in HBSS for 1 h at 37°C. For Fluo-3 wash and Fluo-4 wash experiments, cells were washed 3 times with HBSS, then 200 uL of HBSS was added to each well. The addition consisted of 50 uL (either at 100 nM) (n=3/group).

Comparison of the Calcium 3 Assay Kit with Fluo-4 wash and Fluo-3 wash on FLIPR



CHO-M1 cells were assayed overnight in 100 uL (50,000 cells) on a Costar black wellclear bottom plate. The cells were incubated with 100 uL of either the Calcium 3 Assay Kit, or the medium replaced with either 100 uL of Fluo-4 AM or Fluo-4 AM in HBSS for 1 h at 37°C. For Fluo-3 wash and Fluo-4 wash experiments, cells were washed 3 times with HBSS, then 200 uL of HBSS was added to each well. The addition consisted of 50 uL (either at 100 nM) (n=3/group).

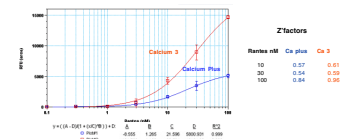
Comparison of the Calcium 3 Assay Kit with Calcium Plus Assay Kit and Fluo-4 wash on FLIPR



CHO-M1 cells were assayed overnight in 100 uL (50,000 cells) on a Costar black wellclear bottom plate. The cells were incubated with 100 uL of either the Calcium 3 Assay Kit, Calcium Plus Assay Kit, or the medium replaced with 100 uL of Fluo-4 AM in HBSS for 1 h at 37°C. For Fluo-4 wash experiments, cells were washed 3 times with HBSS, then 200 uL of HBSS was added to each well. The addition consisted of 50 uL (either at 100 nM) (n=3/group).

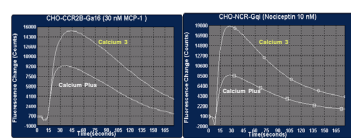
results (continued)

Comparison of the Calcium 3 Assay Kit with Calcium Plus Assay Kit on FLIPR (CHO-CCR5-Gq1 Cells)



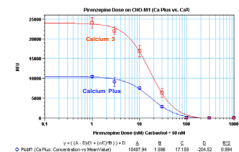
CHO cells transiently transfected with CCR5 and Gq5 were assayed overnight in 100 uL (50,000 cells) on a Costar black wellclear bottom plate. The cells were incubated with 100 uL of either the Calcium 3 Assay Kit or Calcium Plus Assay Kit for 1 h at 37°C. The addition consisted of 50 uL (either at 100 nM) (n=3/group). Data from a representative experiment are shown as mean ± the standard deviation of the mean (n replicates per data point).

Comparison of the Calcium 3 Assay Kit with Calcium Plus Assay Kit on FLIPR (CHO-CCR2B-Ga16 vs. CHO-NCR-Gq)



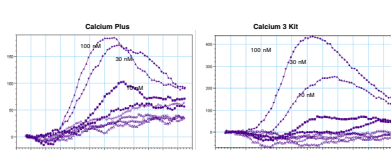
CHO cells transiently transfected with CCR2B and Gq5 or CHO cells transiently transfected with NCR5 and Gq5 (N) were assayed overnight in 100 uL (50,000 cells) on a Costar black wellclear bottom plate. The cells were incubated with 100 uL of either the Calcium 3 Assay Kit or Calcium Plus Assay Kit for 1 h at 37°C. The addition consisted of 50 uL (either at 30 nM (n=3/group), or 50 of Norepinephrine (N) at 10 nM) (n=3/group).

Effect of Pirenzepine on Inhibition of Carbachol-activated Calcium on CHO-M1 Cells. Calcium 3 Assay Kit vs. Calcium Plus Assay Kit on FLIPR



CHO-M1 cells were assayed overnight in 100 uL (50,000 cells) on a Costar black wellclear bottom plate. The cells were incubated with 100 uL of either the Calcium 3 Assay Kit or Calcium Plus Assay Kit for 1 h at 37°C. At the end of 20 minutes after down of the M1 antagonist Pirenzepine were added to the cells. The addition consisted of 50 uL of Carbachol at 50 nM. Data from a representative experiment are shown as mean ± the standard deviation of the mean (n replicates per data point).

Comparison of the Calcium 3 Assay Kit with Calcium Plus Assay Kit on FlexStation (CHO-CCR5-Gq1 Cells)



CHO cells transiently transfected with CCR5 and Gq5 were assayed overnight in 100 uL (50,000 cells) on a Costar black wellclear bottom plate. The cells were incubated with 100 uL of either the Calcium 3 Assay Kit or Calcium Plus Assay Kit for 1 h at 37°C. The addition consisted of 50 uL (either at 100 nM) (n=3/group).

summary

The Calcium 3 Assay Kit is optimized for FLIPR to provide maximum performance with a broad range of GPCR targets and calcium channels.

Benefits

- Homogeneous: Rapid assay development
- Shorter assay time: Increased throughput
- Enhanced signal dynamic range
- Improved data quality, reduced well-to-well variations

Kit formats

Explorer Kit --- 10 vials to make 10 plates (96- or 384-well) includes ready-to-use buffer

Bulk Kit -----10 vials to make 100 plates (96- or 384-well)

Express Kit ----- 2 vials to make 100 Plates (96- or 384-well)

conclusion

The Calcium 3 Assay Kit is a homogenous single-step calcium flux assay that is broadly applicable across a diverse spectrum of biological targets. Its ability to generate very large signal from both weak and robust assay chemistries for different receptor targets. Thus, the Calcium 3 Assay Kit is a "universal" calcium flux assay that will find broad utility in nearly all GPCR screening operations.