ION BIOSCIENCES

Instructions

Brilliant Calcium Gold Assay

Table 1	Kit Contents	*Flex* Kit Cat# 10020-10		*pIONeer* Kit Cat# 10020-2		
Label	Name	Size	Qty	Size	Qty	Storage
Reagent A	Fluo-Gold AM	50 µg Vial	10	50 µg Vial	2	-20° C
Reagent B	DMSO[1]	225 µL Vial	1	Not included in pIONeer		-20° C
Reagent C	50X DySolv	4 mL Bottle	1	800 µL Vial	1	4° C
Reagent D	10X Brilliant Assay Buffer	20 mL Bottle	1	4 mL Bottle	1	4° C
Reagent E	50X TRS[2]	4 mL Bottle	1	800 µL Vial	1	4° C
Reagent F	50X Probenecid[2]	4 mL Bottle	1	800 µL Vial	1	4° C

Description

For the last 25 years, fluorescence-based measures of Ca^{2+} flux have brought about the discovery of small-molecule modulators of a host of ion channels, transporters, GPCRS and other targets of interest for both drug discovery and basic research.

ION Biosciences Brilliant Calcium Gold Assay is a total assay solution for multi-well plate-based, high-throughput measurements of changes in intracellular Ca^{2+} mediated through a wide-variety of plasma membrane and intracellular calcium channels and transporters. The kit includes our Fluo-Gold dye (Ex/Em: 524/552 nm), which is responsive to intracellular Ca^{2+} dynamics. Fluo-Gold's spectral properties enables multiplexing with GFP-expressing cells or other green-fluorescent indicators, and will help mitigate false positives caused by auto-fluorescent compounds within a library. ION Biosciences Brilliant Calcium Gold Assay is also useful for investigating numerous effectors of ion channels and transporters including G protein-coupled receptors, lipid kinases and protein kinases.

Brilliant Calcium Gold Assay provides all the reagents necessary for use as a wash or no-wash assay with adherent or non-adherent cells. The optional use of a probenecid solution and an extracellular background masking solution offers the ultimate in compatibility for cells types which are difficult to load with fluorescent Ca^{2+} indicators (e.g. Chinese Hamster Ovary, CHO cells) and when performing assays in complete, serum-containing cell culture medium is desired.

Getting Started

Before you begin, make sure that you have all the additional reagents and materials you will need for the successful completion of your experiment. While the ION Brilliant Calcium Gold Assay Kit contains all the reagents you will need to prepare your cells for testing, your experiments will likely include other reagents which are not included in your ION Brilliant Calcium Gold Assay Kit. Notably compounds to be tested are not included, neither are buffers and solvents for the dissolution of those compounds. The Brilliant Calcium Gold Assay Kit also does not contain reagents necessary for cell

Getting Started (Continued)

culture. ION's pIONeer Kits do not contain DMSO for solubilizing the dye. In addition to reagents, a fluorescence plate reader that is capable of providing excitation at ~530 nm and collecting emission at ~550 nM is required. Ideally this plate reader will be able to collect kinetic data at an interval of once per second (1 Hz). Examples of plate readers of this type are the WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR and Molecular Devices FlexStation.

Laboratory Procedures

Wash Method – Adherent Cells

The instructions given below are for one, 384-well microplate. Certain aspects of the instructions may need to be altered, as appropriate, for multiple microplates or other assay formats (e.g. 96-well microplates or non-adherent cells). The Fluo-Gold AM and Fluo-Gold AM -containing solutions should be protected from direct light.

1. Add 20 µL DMSO[1] (Reagent B) to the tube containing Fluo-Gold AM (Reagent A).
2. Vortex until Reagent A is fully dissolved.
3. Add appropriate volume of water (Table 2) to a 15 mL centrifuge tube.
4. Add 1 mL of 10X Brilliant Assay Buffer (Reagent D) to tube from step 3.
5. Add 200 µL of DySolv (Reagent C) to the tube from step 4.
6. If desired add 200 µL of Probenecid Solution (Reagent F) to the tube from step 5.
7. Add 20 µL of Fluo-Gold AM Solution from step 2 to the tube from step 6.
8. Briefly vortex the tube from step 7 to mix.

Procedure Continues on Next Page

Table 2 Dye Loading Solution (Wash Method)

Label	Name	Method A	Method B
Reagent A+B	Fluo-Gold AM Solution	20 µL	20 µL
Reagent C	50X DySolv	200 µL	200 µL
Reagent D	10X Brilliant Assay Buffer	1 mL	1 mL
Reagent F	50X Probenecid[2]	-	200 µL
	Water	8.8 mL	8.6 mL
	Total	10 mL	10 mL

9. Remove the cell-culture medium from the 384-well microplate containing the cells of interest.
10. Add 20 µL per well of the Dye Loading Solution from step 8 to the microplate from step 9.
11. Incubate the microplate containing the cells and Dye Loading Solution for 30 - 60 minutes at 37° C.
12. Prepare Wash Solution in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X Brilliant Assay Buffer (Reagent D) and other components if desired as shown in Table 3.
13. Briefly vortex the tube from step 12 to mix.

Table 3 Wash Solution

Label	Name	Method A	Method B	Method C	Method D
Reagent D	10X Brilliant Assay Buffer	1 mL	1 mL	1 mL	1 mL
Reagent E	50X TRS[2]	-	200 µL	-	200 µL
Reagent F	50X Probenecid[2]	-	-	200 µL	200 µL
	Water	9 mL	8.8 mL	8.8 mL	8.6 mL
	Total	10 mL	10 mL	10 mL	10 mL

14. Remove Dye Loading Solution from microplate in step 11.
15. Add 20 µL per well of the Wash Solution prepared in step 13 to the microplate from step 14.
16. Transfer the washed, dye-loaded, cell-containing microplate from step 15, along with an additional microplate containing a stimulus solution of interest, to a kinetic-imaging plate reader (e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR or Molecular Devices FlexStation). See Table 5 for recommended instrument settings.
17. Acquire data using an excitation wavelength of ~530 nm, an emission wavelength of ~550 nm and an acquisition frequency of 1 Hz. Begin data acquisition and after 10 seconds, add appropriate volume of the stimulus solution to the cell-containing plate and continue data acquisition for an additional 90 seconds[3].

No-wash Method – Adherent Cells

1. Add 20 µL DMSO[1] (Reagent B) to the tube containing Fluo-Gold AM (Reagent A)
2. Vortex until Reagent A is fully dissolved.
3. Add appropriate volume of water (Table 4, next page) to a 15 mL centrifuge tube.
4. Add 1 mL of 10X Brilliant Assay Buffer (Reagent D) to tube from step 3.
5. Add 400 µL of DySolv (Reagent C) to the tube from step 4.
6. Add 400 µL of TRS (Reagent E) to the tube from step 5.
7. If desired add 400 µL of Probenecid Solution (Reagent F) to the tube from step 6.
8. Add 20 µL of Fluo-Gold AM Solution from step 2 to the tube from step 7.
9. Briefly vortex the tube from step 8 to mix.

Procedure Continues on Next Page

Table 4	Dye Loading Solution (No-wash Method)		
Label	Name	Method A	Method B
Reagent A+B	Fluo-Gold AM Solution	20 µL	20 µL
Reagent C	50X DySolv	400 µL	400 µL
Reagent D	10X Brilliant Assay Buffer	1 mL	1 mL
Reagent E	50X TRS[2]	400 µL	400 µL
Reagent F	50X Probenecid[2]	-	400 µL
	Water	8.2 mL	7.8 mL
	Total	10 mL	10 mL

10. Add 20 µL per well of the Dye Loading Solution from step 9 to the cell-containing microplate. Do not remove the cell culture medium.
11. Incubate the microplate containing the cells and Dye Loading Solution for 30 - 60 minutes at 37° C in a cell culture incubator.
12. Transfer the dye-loaded, cell-containing microplate from step 11, along with an additional microplate containing a stimulus solution of interest, to a kinetic-imaging plate reader (e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR or Molecular Devices FlexStation). See Table 5 for recommended instrument settings.
13. Acquire data using an excitation wavelength of ~530 nm, an emission wavelength of ~550 nm and an acquisition frequency of 1 Hz. Begin data acquisition and after 10 seconds add appropriate volume of the stimulus solution to the cell-containing plate and continue data acquisition for an additional 90 seconds[3].

Table 5	Recommended Instrument Settings
Setting	Recommendation
Read Mode	'Bottom' read mode only
Ex/Em wavelengths[4]	~517 nm/546 nm
Cutoff wavelength	530 nm
Filter selection	YFP
Contact support@ionbiosciences.com for additional recommendations and guidance on optimizing your application.	

Laboratory Procedures - Footnotes

[1] DMSO is hygroscopic and should be stored tightly closed at -20° C with desiccant pack to prevent the solvent from becoming wet. Wet solvent causes difficulties with dissolution of the dye. Use the DMSO within 6 months of receipt. ION's pIONeer Kits do not contain DMSO.

[2] Caution is advised when using Probenecid and/or TRS as they may have undesirable effects on assay performance for the target of interest. Probenecid may be included in the Dye Loading Solution to aid in dye retention. This may be particularly important in certain cell lines (e.g. CHO cells). TRS contains a membrane-impermeant dye useful for masking extracellular fluorescence.

[3] The timing and volume of stimulus solution addition may vary. Some experiments may include the addition of other solutions to the cell-containing microplate prior to the addition of the stimulus solution. In these cases, the volume of the stimulus solution addition should be altered to account for the additional volume of solution in the cell-containing microplate.

[4] To prevent bleed-through or spectral overlap, the Ex/Em wavelengths may need to be optimized by broadening the interval between the wavelengths. Results generated on a Molecular Devices Flexstation 3.

Additional Information

Additional dye indicator and buffer reagents can be purchased either directly from our website or by contacting our Sales Department. Custom and bulk sizes are also available. Contact Sales for more information.

Table 6	Additional Reagents	Available Sizes	
Kit Label	Name	Size	Catalog #
Reagent A	Fluo-Gold AM	500 µg x 1 Vial	1045C
		50 µg x 10 Vials	1045F
		50 µg x 3 Vials	1045G
Reagent C	50X DySolv	20 mL Bottle	7501A
Reagent D	10X Brilliant Assay Buffer	10 mL Bottle	7010X
Reagent E	50X TRS	20 mL Bottle	7060A
Reagent F	50X Probenecid	20 mL Bottle	7300P-50