ION BIOSCIENCES

Instructions

FLUO-GOLD AM

Materials Needed

Name	Volume	Containers	Storage
Fluo-Gold AM (50 µg vial)	Dry	1	-20° C
DMSO	25 µL	1	25° C
100X Pluronic F-127 solution	100 µL	1	4° C
100X Probenecid solution (optional)	100 µL	1	4° C
100X TRS solution (optional)	100 µL	1	4° C

Description

Fluo-Gold is a gold fluorescent (Ex/Em = 528/548 nm), intracellular calcium indicator. Designed to measure intracellular Ca^{2+} flux in high throughput screening and fluorescence microscopy applications. Fluo-Gold's red-shifted excitation and emission enable multiplexing with GFP-expressing cells or other green fluorescent indicators and minimizes interference from auto-fluorescent compounds.

Laboratory Procedures

The following protocol provides general guidelines for using this dye to measure intracellular calcium. All loading conditions (dye concentration, temperature, and time) should be optimized for your specific assay, application, and instrumentation.

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 10 mL of assay buffer to a conical tube (15 – 50 mL). HEPES-buffered Hank's Balanced Salt Solution (pH = 7.2 – 7.4) is the most used assay buffer, although other buffers can also be used. We recommend using our 1X Brilliant Calcium assay buffer (Catalog #: 7010d) with this product.
3. Add 100 µL of 100X Pluronic F-127 solution (Catalog #: 7601A) to conical tube. Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.
4. (Optional) Add 100 µL of 100X Probenecid solution (Catalog #: 7300A) to conical tube. Probenecid is an anion transport inhibitor that improves intracellular dye retention. Although it is not required for all cell types and dyes, it is recommended in most cases to optimize assay performance.[1]
5. (Optional) Add 100 µL of TRS solution (Catalog #: 7060A). TRS is a membrane impermeant dye useful for masking extracellular fluorescence.[1]
6. Vortex conical tube briefly to mix.

[1]Caution is advised when using Probenecid and/or TRS as they may have undesirable effects on assay performance for the target of interest.

Laboratory Procedures (continued)

7. Dissolve Fluo-Gold AM in 25 µL of DMSO. After adding DMSO, vortex tube briefly to dissolve the indicator dye, then centrifuge briefly to collect all contents at the tube bottom. Add entire contents of indicator dye tube to assay buffer solution to make a dye loading solution.[2]
8. Vortex dye loading solution briefly to mix.
9. Remove the cell culture medium and add dye loading solution. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 µL; 384-well plate, 20 µL.[3]
10. Incubate in a cell culture incubator at 37°C for 30 - 60 minutes.
11. 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.

[2]The dye loading solution should be used within 2 hours of dye addition for best results.

[3]In some cases, a no wash format works best. If a no wash format is indicated for your application, we recommend doubling the concentration of all reagents in your dye loading buffer.

[4]To minimize extracellular background, dye loading solution can be replaced with assay buffer containing 1X probenecid solution (optional) and/or 1X TRS solution (optional).

Example Results

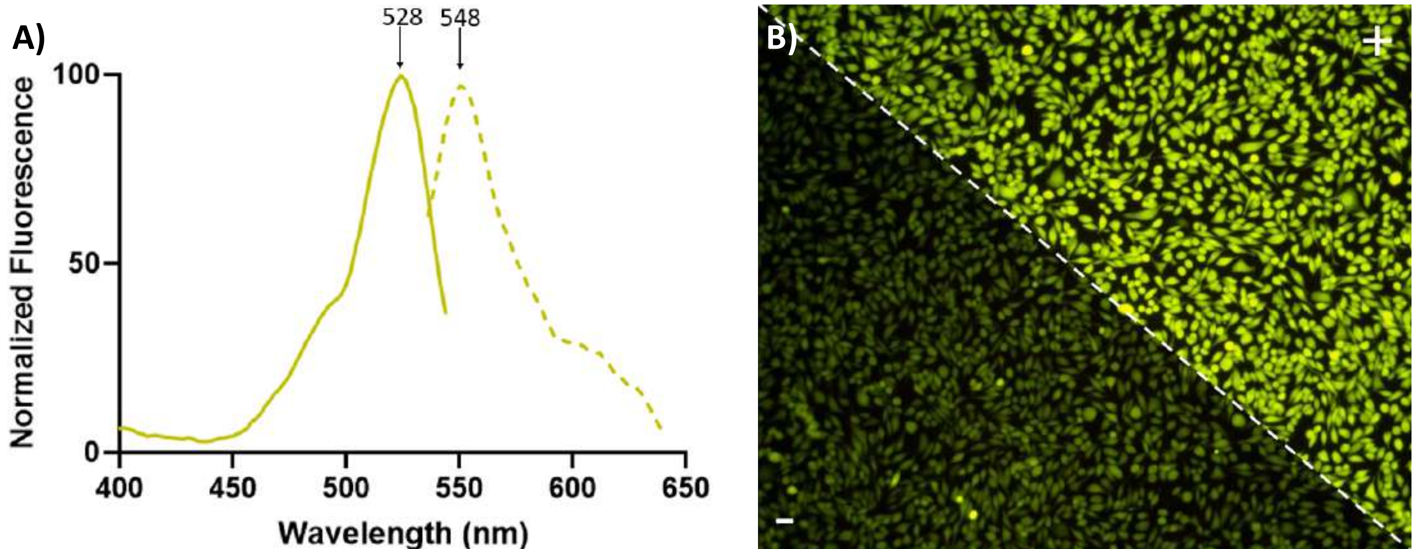

Figure 1. Fluo-Gold spectral properties. A) Spectral data was collected from CHO K1 cells loaded with Fluo-Gold for 30 minutes. Data was acquired with a BioTek® Cytation 5 plate reader. Maximum excitation is at 528 nm and maximum emission is at 548 nm. B) Cells were imaged using a Zeiss Axiovert 25 Inverted Phase Contrast Microscope, before (-) and after (+) the addition of 100nM ionomycin, a calcium ionophore. (Ex: 517/20 nm, Em: 575/59 nm).

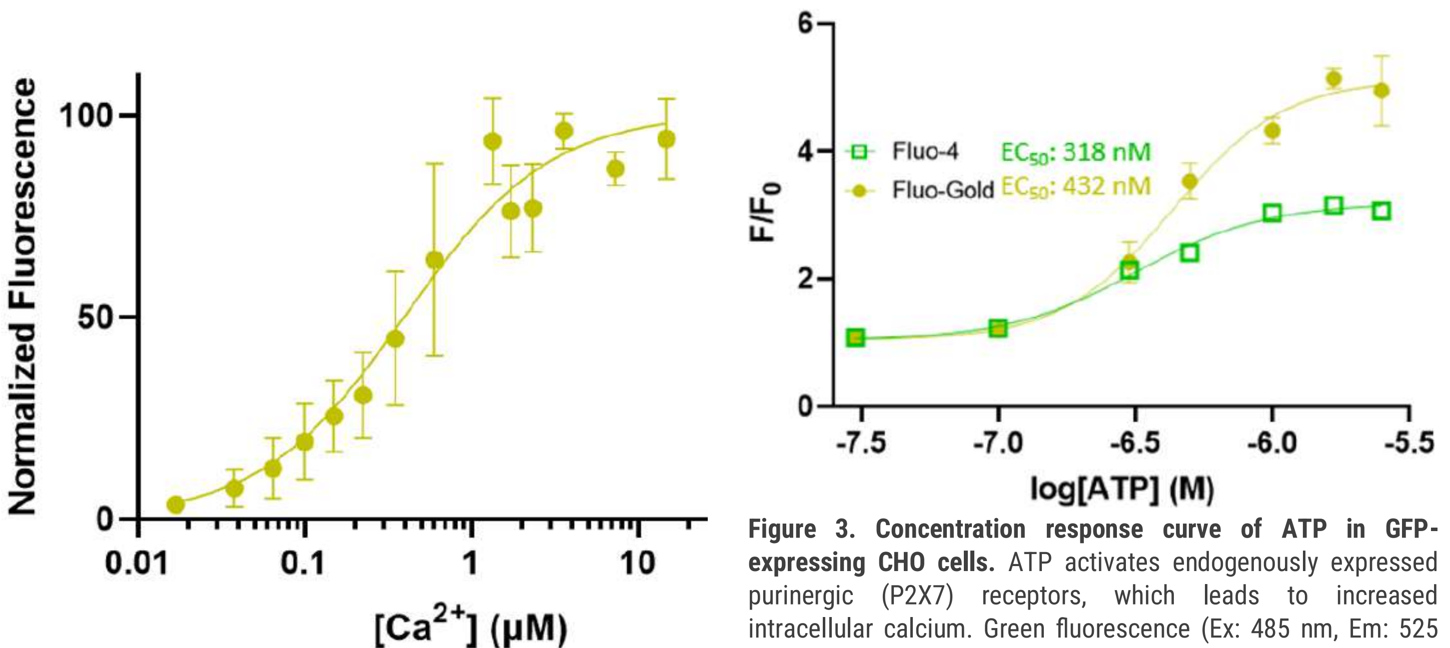

Figure 2. Saturation binding curve. Fluo-Gold titrated in calcium calibration buffers. Fluo-Gold has a slightly lower calcium affinity (K_d ~400 nM) than Fluo-4.

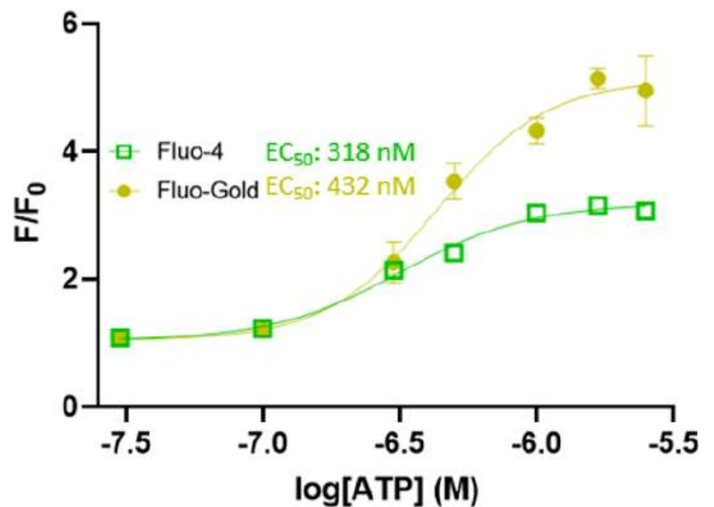

Figure 3. Concentration response curve of ATP in GFP-expressing CHO cells. ATP activates endogenously expressed purinergic (P2X7) receptors, which leads to increased intracellular calcium. Green fluorescence (Ex: 485 nm, Em: 525 nm, Cutoff: 515 nm) and gold fluorescence (Ex: 517 nm, Em: 546 nm, Cutoff: 530 nm) were recorded at ~1 Hz using a Molecular Devices FlexStation® plate reader. Error bars represent standard deviation (n=3). Fluo-Gold displays a larger dynamic range and is better suited for assays using GFP-expressing cells.

Example Results

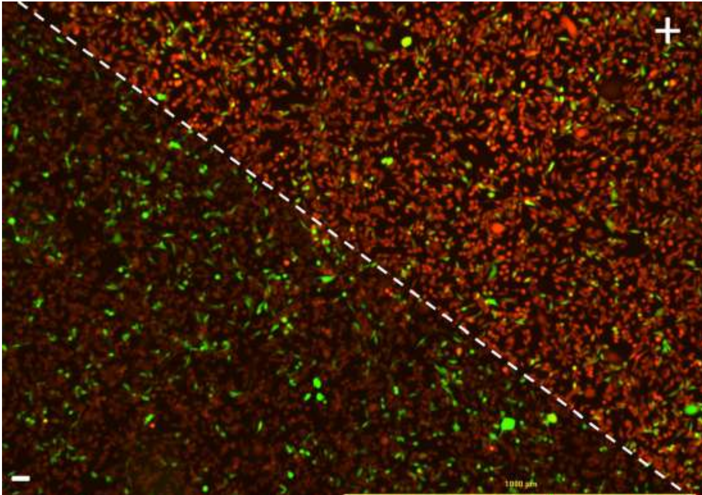

Figure 4. Calcium mobilization in GFP-expressing cells loaded with Fluo-Gold. Representative fluorescence images of polyclonal, GFP-expressing CHO cells loaded with Fluo-Gold for ~30 minutes. Images were acquired before (-) and after (+) addition of the ionomycin (100 nM) with a BioTek® Cytation equipped with a GFP filter cube (Ex: 469/35 nm, Em: 529/39 nm), Propidium Iodide filter cube (Ex: 531/40 nm, Em: 647/57 nm), and 4X objective. Fluo-Gold fluorescence appears red because a propidium iodide filter cube was used to capture the images.

Figure 5. Fluo-Gold minimizes compound interference from green fluorescent compounds. CHO K1 cells were loaded with Fluo-Gold for 30 minutes. Fluorescence for Fluo-Gold (Ex: 540 nm, Em: 580 nm, Cutoff: 570 nm) was recorded at ~1 Hz using a Molecular Devices FlexStation®. Baseline fluorescence was collected for 30 seconds before the addition of 5 μM carboxyfluorescein (FAM), a highly fluorescent compound, and 5 μM FAM + 1.7 μM ATP. ATP activates endogenously expressed P2X7 receptors, which leads to increased intracellular calcium. FAM fluorescence does not disrupt Fluo-Gold signal. Inset) Fluorescence profile of 5 μM FAM addition when using acquisition settings for Fluo-4 (Ex: 485 nm, Em: 525 nm, Cutoff: 515 nm).