ION BIOSCIENCES

Instructions

BAPTA JF™549

Materials Needed

Name	Volume	Containers	Storage
BAPTA JF549 (50 µg vial)	Dry	1	-20° C
DMSO	25 µL	1	-20° C
100X Pluronic F-127 solution	100 µL	1	4° C
100X Probenecid solution (optional)	100 µL	1	25° C

Description

BAPTA JF549 is a red fluorescent (Ex/Em = 546/569 nm), intracellular calcium indicator. Designed with a Janelia Fluor® dye backbone, it displays excellent brightness and photostability for measuring fast calcium dynamics in neurons and cardiomyocytes. The red-shifted fluorescence properties help minimize tissue autofluorescence, and also enable multicolor imaging and the use of optogenetic tools for triggering calcium transients.

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. Vortex conical tube briefly to mix.

[1]Caution is advised when using Probenecid as it may have undesirable effects on assay performance for the target of

Laboratory Procedures (continued)

7. Dissolve BAPTA JF549 in 25 µL of DMSO. After adding DMSO, vortex tube briefly to dissolve the dye, then centrifuge briefly to collect all contents at the tube bottom. Add entire contents of 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 37ºC for 30 minutes.
11. Conduct a wash step to remove the dye loading solution and replace with cell culture medium or assay buffer. Repeat, if necessary, to completely remove extracellular dye.
12. Acquire data using a fluorescence microscope equipped with Cy3 or TRITC filters or a fluorescence plate reader excitation using an excitation wavelength of ~545 nm, an emission wavelength of ~570 nm and an acquisition frequency of 1-10 Hz.

[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.

References

1. Deo, Claire, et al. "Isomeric tuning yields bright and targetable red Ca2+ indicators." *Journal of the American Chemical Society 141.35 (2019): 13734-13738.*