Instructions

SBFI AM

Materials Needed			
Name	**Volume**	**Containers**	**Storage**
SBFI 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

Description

SBFI is a UV-excitable, ratiometric green indicator for intracellular potassium (Na^+) measurements. Ex/Em: 340/505 nm can be used to measure Na^+-bound SBFI, and Ex/Em: 380/505 nm can be used to detect Na^+-free SBFI. It is ~18X more selective for Na^+ over K^+. Ratiometry is optimal for imaging applications where quantification of intracellular Na^+ concentrations is desired, and reduces effects of photobleaching, heterogenous dye loading, and variable cell morphology.

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 interest.

Laboratory Procedures (continued)

6. Dissolve SBFI 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]
7. Vortex dye loading solution briefly to mix.
8. 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]
9. Incubate in a cell culture incubator at 37ºC for 60 minutes.
10. Read fluorescence using a plate reader (Ex/Em: 340 or 380 nm/505 nm) or image using a fluorescence microscope (using filters for Fura).[4,5]

[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).