Protocol Booklet

Product Code(s)	HB24669
Product Name(s)	BAPTA-AM Janelia Fluor® 549
Purpose	Measurement of intracellular Ca^{2+} in cultured cells

Please note: This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

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Product Overview

BAPTA-AM Janelia Fluor® 549 is a membrane permeable, red-shifted (Excitation 546nm, Emission 569nm), intracellular calcium (Ca^{2+}) indicator (K_d = 310nM). It is suitable for measurement of fast calcium dynamics in neurons and cardiomyocytes with excellent photostability and brightness compared to genetically encoded sensors. There are reduced issues with tissue autofluorescence and background fluorescence due to the red-shifted fluorophore. BAPTA-AM Janelia Fluor® 549 is compatible with fluorescence microscopy using TRITC or Cy3 filters.

Components & Storage

BAPTA-AM Janelia Fluor® 549 is provided as either:

SKU	Component	Quantity	Storage Temperature
HB24669	BAPTA-AM Janelia Fluor® 549	500µg	-20°C
		10x50µg	

This protocol additionally requires:

Component	Quantity	Storage Temperature
DMSO	25µl	RT
Pluronic F-127	10mg	4°C
Probenecid	7.7mg	4°C
Assay Buffer (HEPES-buffered Hank's Balanced Salt Solution (pH = 7.3)*	10ml	RT

* Please see recipe at the end of this protocol book.

Protocol

The following protocol provides general guidelines for using BAPTA-AM Janelia Fluor® 549 to measure intracellular calcium in cultured cells. All loading conditions (dye concentration, temperature, and time) should be optimized for your specific assay, application, and instrumentation.

1. Culture cells following standard protocols to approximately 80-100% confluence.
2. Prepare the loading solution freshly following the below table, vortex well and use within 2 hours.
3. Remove the cell culture medium, briefly wash in plain media (without serum), then add dye loading solution. Recommend volumes are:
    a. 35mm dish / 6-well plate - 1.5 mL/well,
    b. 96 well plate - 100 µL/well,
    c. 384 well plate - 20 µL/well,
4. Incubate in a cell culture incubator at 37ºC for 30 minutes.
5. Wash cells twice in fresh assay buffer
6. Read fluorescence using either a plate reader (Excitation: 545nm, Emission 575nm) or image using a fluorescence microscope using a compatible filter set (e.g. Cy3, TRITC).

Recipes

BAPTA-AM Janelia Fluor® 549 Loading Solution

Component	Concentration	Quantity	Notes
BAPTA-AM Janelia Fluor® 549	4.1µM	50µg	Dissolve in DMSO then aliquot and store any unused dye at -20°C
Assay Buffer	1X	10ml	Normally HEPES buffered HBSS but other buffers have been also successfully used.
Pluronic F-127	0.1%	10mg	Surfactant that helps the dissolution of dye therefore ensuring even dye distribution and cellular loading.
Probenecid	2.7mM	7.7mg	Anion transport inhibitor that improves intracellular dye retention. Not required for all cell types, it is recommended in most cases to optimize assay performance.

Please note: Combine components then vortex thoroughly. Use within 2 hours of creation. Do not freeze.

HEPES-buffered Hank's Balanced Salt Solution (Assay Buffer)

Component	MW (g/mol)	g/L	Concentration (mM)
Calcium Chloride	110.98	0.14	1.26
Magnesium Chloride Hexahydrate	203.30	0.1	0.49
Magnesium Sulfate Heptahydrate	246.47	0.1	0.41
Potassium Chloride	74.55	0.4	5.33
Potassium Phosphate Monobasic	136.09	0.06	0.44
Sodium Bicarbonate	84.01	0.35	4.17
Sodium Chloride	58.44	8	138.00
Sodium Phosphate Dibasic	141.96	0.048	0.34
D-Glucose (Dextrose)	180.16	1	5.56
HEPES	238.30	4.76	20.00

Please note: Add all components to dH_2O, mix well then adjust to pH 7.3

Guidelines, precautions, troubleshooting

Please contact our technical support team at technicalhelp@hellobio.com for advice on how to resolve any problems encountered when using this product. Observe safe laboratory practice and consult the safety datasheet. Please see the datasheet on our website for general guidelines, precautions, limitations on the use of the product.

Contact

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