BAPTA Janelia Fluor® 549 HaloTag® Ligand (BAPTA JF549 HTL)

Table 1 - Materials Needed	Most Items Available from ION Biosciences	
Name	**Volume**	**Description/Purpose**
BAPTA JF549 HTL (50 µg vial)	Dry - 1 Vial	Membrane Permeable Calcium Dye Indicator
DMSO[1]	25 µL	Solvent for Dissolution of Dye
100X Pluronic F-127 solution[2]	100 µL	Biocompatible Surfactant for Dye Loading
100X Probenecid solution (optional)[3]	100 µL	Intracellular Dye Retention Aid
50X TRS (optional)[4]	200 µL	Extracellular Fluorescence Masking Agent
Assay Buffer - We Suggest One of the Following:		
⇒ 1X HEPES-Buffered Hank's Balanced Salt Solution	10 mL	1X - Ready to Use Assay Buffer
⇒ 10X Brilliant Assay Buffer	1 mL	10X - Concentrated Assay Buffer
Water	As Needed	Dilution of 10X Assay Buffer (if used)

Description

BAPTA Janelia Fluor 549 HaloTag Ligand (BAPTA JF549 HTL) is a red fluorescent (Ex/Em = 550/575 nm) intracellular calcium (Ca^{2+}) indicator that displays a chloroalkane ligand for binding to HaloTag. The HaloTag ligand enables localization to specific cell phenotypes in mixed cell populations and intracellular organelles using HaloTag technology. Designed with a Janelia Fluor dye backbone, the calcium indicator displays excellent brightness and photostability for measuring fast calcium dynamics in neurons and cardiomyocytes. It also outperforms red-shifted, genetically encoded calcium sensors in sensitivity and fluorescence intensity. The red-shifted fluorescence minimizes tissue autofluorescence, enables multicolor imaging, and permits the use of optogenetic tools for triggering calcium transients. ***This dye requires the use of cells transiently or stably expressing HaloTag to work.** For more information about HaloTag technology, visit

General Considerations

The following protocol provides general guidelines for using this dye to measure intracellular calcium flux. All loading conditions (dye concentration, temperature, and time) should be optimized for your specific assay, cell type, and application. ***This dye requires the use of cells transiently or stably expressing HaloTag to work.**

Laboratory Procedures - Dye Load with Wash Protocol

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 25 µL DMSO[1] to the tube containing BAPTA JF549 HTL.
3. Vortex until BAPTA JF549 HTL is fully dissolved. Centrifuge briefly to collect all contents at the tube bottom.
4. Add the appropriate volume (see **Table 2**, below) of 1X Assay Buffer to a 15 mL conical tube. If using 10X Assay Buffer, add the appropriate volume of Water (**Table 3**, below) and 1 mL of 10X Assay Buffer to a 15 mL conical tube.
5. Add 100 µL of 100X Pluronic F-127[2] solution to the conical tube from **step 4**.
6. (Optional) Add 100 µL of 100X Probenecid[3] solution to the conical tube from **step 5**.
7. (Optional) Add 200 µL of 50X TRS[4] solution to the conical tube from **step 6**.
8. Vortex conical tube from **step 7** briefly to mix.

Table 2 Dye Loading Solution[5]	**Using 1X Assay Buffer**			
Name	**Method A**	**Method B**	**Method C**	**Method D**
BAPTA JF549 HTL in DMSO[1] Solution	25 µL	25 µL	25 µL	25 µL
100X Pluronic F-127[2] solution	100 µL	100 µL	100 µL	100 µL
100X Probenecid[3] solution (optional)	100 µL	100 µL	-	-
50X TRS[4] (optional)	200 µL	-	200 µL	-
1X HEPES-Buffered Hanks Balanced Salt Solution	9.6 mL	9.8 mL	9.7 mL	9.9 mL
Total	10 mL	10 mL	10 mL	10 mL

Table 3 Dye Loading Solution[5]	**Using 10X Assay Buffer**			
Name	**Method A**	**Method B**	**Method C**	**Method D**
BAPTA JF549 HTL in DMSO[1] Solution	25 µL	25 µL	25 µL	25 µL
100X Pluronic F-127[2] solution	100 µL	100 µL	100 µL	100 µL
100X Probenecid[3] solution (optional)	100 µL	100 µL	-	-
50X TRS[4] (optional)	200 µL	-	200 µL	-
10X Brilliant Assay Buffer	1 mL	1 mL	1 mL	1 mL
Water	8.6 mL	8.8 mL	8.7 mL	8.9 mL
Total	10 mL	10 mL	10 mL	10 mL

Procedure Continues on Next Page

Laboratory Procedures - Dye Load with Wash Protocol (continued)

9. Add the entire contents of the BAPTA JF549 HTL in DMSO solution from **step 3** to the conical tube from **step 8** to make the **Dye Loading Solution**[5].
10. Vortex the **Dye Loading Solution**[5] from **step 9** briefly to mix.
11. Remove the cell culture medium and add the **Dye Loading Solution**[5] from **step 10**. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 µL; 384-well plate, 20 µL.
12. Incubate in a cell culture incubator at 37ºC for 60 minutes.
13. Prepare 10 mL of **Wash Solution**[6] (**Table 4** below) in a 15 mL centrifuge tube by adding the appropriate amounts of assay buffer and water.
14. Prepare 10 mL of **Imaging Solution**[7] (**Table 5** below) in a 15 mL centrifuge tube by adding the appropriate amounts of assay buffer, 50X TRS[4], and water.

Table 4 Wash Solution[6]

Name	Method A	Method B
1X HEPES-Buffered Hanks Balanced Salt Solution	10 mL	-
10X Brilliant Assay Buffer	-	1 mL
Water	-	9 mL
Total	10 mL	10 mL

Table 5 Imaging Solution[7]

Name	Method A	Method B
1X HEPES-Buffered Hanks Balanced Salt Solution	9.8 mL	-
50X TRS[4]	200 µL	200 µL
10X Brilliant Assay Buffer	-	1 mL
Water	-	8.8 mL
Total	10 mL	10 mL

15. After the 60 minute incubation period (**step 12**), remove the **Dye Loading Solution**[5] and wash the wells with the **Wash Solution**[6] prepared in **step 13**.
16. Remove the **Wash Solution**[6] and add **Imaging Solution**[7] prepared in **step 14** to the wells and then incubate at 37ºC for 60 minutes to allow excess dye to efflux before conducting the assay.

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Laboratory Procedures - Dye Load with Wash Protocol (continued)

17. Transfer the washed, dye-loaded, cell-containing microplate from **step 16** to an imaging fluorescence microscope (using Cy3 or TRITC filters) or a fluorescence plate reader (e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR, Molecular Devices FlexStation, or BioTek Cytation 5). Acquire data using an excitation wavelength of ~ 550 nm, an emission wavelength of ~ 575 nm and an acquisition frequency of 1 Hz. See **Table 6** for recommended instrument settings.

Table 6	**Recommended Instrument Settings**
Setting	**Recommendation**
Read Mode (Plate Readers)	'Bottom' read mode only
Ex/Em wavelengths[8]	~550 nm/575 nm
Cutoff wavelength	570 nm
Filter selection	Cy3, TRITC
Contact support@ionbiosciences.com for additional recommendations and guidance on optimizing to your application.	

Laboratory Procedures - Footnotes

1 DMSO is hygroscopic and should be stored tightly closed. Wet solvent causes difficulties with dissolution of the dye.

2 Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.

3 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, and may be particularly important to include for some cell lines (e.g. CHO cells).

4 TRS is a membrane impermeant dye useful for masking extracellular fluorescence. Caution is advised when using TRS or any other extracellular masking solutions as they may have undesirable effects on assay performance for the target of interest.

5 The Dye Loading Solution should be used within 2 hours of dye addition for best results.

6 To maximize results, a plate-washing instrument can be used to thoroughly wash the wells of excess dye. Scale the volumes of components to those required to operate the plate-washing instrument. Contact sales@ionbiosciences.com to inquire about bulk purchases of our assay buffers to use with your instrument.

7 In some cases, a no-wash format may work best. If a no-wash format is indicated for your application, we recommend doubling the concentrations of 100X Pluronic, 100X Probenecid, and 50X TRS in the dye-loading buffer.

8 To prevent bleed-through or spectral overlap, the Ex/Em wavelengths may need to be optimized by broadening the interval between the wavelengths.

Additional Information

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 4	Available Reagents	Available Sizes	
Name		**Size**	**Catalog #**
BAPTA Janelia Fluor 549 HaloTag Ligand (BAPTA JF549 HTL)		500 µg x 1 Vial 50 µg x 10 Vials 50 µg x 3 Vials	1049-HTL-C 1049-HTL-F 1049-HTL-G
100X Pluronic F-127 Solution		10 mL Bottle	7601A
100X Probenecid Solution		10 mL Bottle	7300P-100
50X TRS Solution		20 mL Bottle	7060A
1X HEPES-Buffered Hank's Balanced Salt Solution (1X HHBSS)		100 mL Bottle	7001
10X Brilliant Assay Buffer		10 mL Bottle	7010X