BCECF AM

Table 1 - Materials Needed	Most Items Available from ION Biosciences	
Name	**Volume**	**Description/Purpose**
BCECF AM (50 µg vial)	Dry - 1 Vial	Membrane Permeable Intracellular pH 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
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 Thallium Assay Buffer	1 mL	10X - Concentrated Assay Buffer
Water	As Needed	Dilution of 10X Assay Buffer (if used)

Description

BCECF AM is the most popular green fluorescent, intracellular pH indicator. BCECF has a pKa of ~7, and exhibits pH-dependent, dual-excitation properties (Ex/Em 430nm/535nm and 490nm/535nm) for ratiometric analysis. Ratiometry is optimal for imaging applications, and reduces effects of photobleaching, heterogenous dye loading, and variable cell morphology. For HTS applications, BCECF can also be used in non-ratiometric mode using standard fluorescein excitation and emission settings.

Laboratory Procedures

The following protocol provides general guidelines for using this dye to measure intracellular pH. 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 25 µL DMSO[1] to the tube containing BCECF AM.
3. Vortex until BCECF AM is fully dissolved. Centrifuge briefly to collect all contents at the tube bottom.
4. Add the appropriate volume (see **Table 2**, next page) of 1X Assay Buffer to a 15 mL conical tube. If using 10X Assay Buffer, add the appropriate volume of Water (**Table 2**) 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**.

Procedure Continues on Next Page

Laboratory Procedures (continued)

6. (Optional) Add 100 µL of 100X Probenecid[3] solution to the conical tube from **step 5**.
7. Vortex conical tube from **step 6** briefly to mix.
8. Add the entire contents of the BCECF AM in DMSO solution from **step 3** to the conical tube from **step 7** to make the **Dye Loading Solution**[4].
9. Vortex the **Dye Loading Solution**[4] from **step 8** briefly to mix.

Table 2 Dye Loading Solution[4]	1X Assay Buffer		10X Assay Buffer	
Name	**Method A**	**Method B**	**Method C**	**Method D**
BCECF AM 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	100 µL	-	100 µL	-
1X HEPES-Buffered Hanks Balanced Salt Solution	9.8 mL	9.9 mL	-	-
10X Brilliant Assay Buffer	-	-	1 mL	1 mL
Water	-	-	8.8 mL	8.9 mL
Total	10 mL	10 mL	10 mL	10 mL

10. Remove the cell culture medium and add the **Dye Loading Solution**[4] from **step 9**. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 µL; 384-well plate, 20 µL.[5]
11. Incubate in a cell culture incubator at 37ºC for 60 minutes.
12. Conduct a wash[5] step to remove the **Dye Loading Solution**[4] and replace with cell culture medium or assay buffer. Repeat, if necessary, to completely remove extracellular dye.
13. Acquire data using a fluorescence microscope equipped with BCECF or FITC filters or a fluorescence plate reader using an excitation wavelength of ~430 or 490 nm, an emission wavelength of ~535 nm and an acquisition frequency of 1 Hz[6]. See **Table 3** below for recommended settings.

Table 3 Recommended Instrument Settings	
Setting	**Recommendation**
Read Mode (Plate Readers)	'Bottom' read mode only
Ex/Em wavelengths[6]	~430 or 490 nm/535 nm
Filter selection	BCECF (ratiometric analysis), FITC (non-ratiometric imaging)
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.

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

[5] 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 100X Pluronic F-127 and 100X Probenecid in your dye loading buffer.

[6] 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 #
BCECF AM		1 mg x 1 Vial 50 µg x 20 Vials	4011B 4011E
100X Pluronic F-127 Solution		10 mL Bottle	7601A
100X Probenecid Solution		10 mL Bottle	7300P-100
1X HEPES-Buffered Hank's Balanced Salt Solution (1X HHBSS)		100 mL Bottle	7001
10X Brilliant Thallium Assay Buffer		10 mL Bottle	7010T