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Protocol for Loading BCECF, AM *UltraPure grade* Into Live Cells

IMPORTANT DISCLAIMER: The following is a sample protocol for loading BCECF, AM *UltraPure grade* esters into live cells. This protocol only provides a guideline and should be modified according to your experimental needs. Please read the entire protocol before starting.

How to use this protocol:

First, select your microplate format and enter in the required specifications. Next, follow the instructions provided in each section to prepare the necessary buffers, stock solutions, and working solutions needed to successfully load your cells with BCECF, AM *UltraPure grade*. For assistance, use tools and calculators to determine the amount of component required for each part of the loading procedure.

Key parameters

Instrument:	Fluorescence microplate reader
Excitation	490, 430
Emission	535
Cutoff	515
Recommended plate	Solid black

Instrument:	Fluorescence microscope
Excitation	FITC
Emission	FITC
Recommended plate	Black wall/clear bottom

Select your microplate format

Black wall/clear bottom microplate:	96-wells
Enter the number of wells to be used:	32
Volume of culture medium per well [1]:	100 µL
Volume of working solution per well [1]:	100 µL

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Prepare these materials

IMPORTANT NOTE: This protocol includes the non-ionic detergent Pluronic® F-127 and the organic anion-transport inhibitor probenecid. Both reagents are not required, but highly recommended. To remove a reagent from the loading protocol, uncheck the appropriate box:

Required

☑ BCECF, AM *UltraPure grade*

☑ Hanks and Hepes Buffer *(HHBS) or a buffer of your choice

☑ 100% DMSO

Optional

☑ 10% Pluronic® F-127

Step-by-step guide:

1. Prepare an HHBS buffer, and a 10% Pluronic® F-127 solution.

a. For instructions on how to prepare a HHBS buffer, see our buffer recipe page
b. For instructions on how to prepare a 10% Pluronic® F-127 solution, see recipe

2. Prepare a 2 mM to 20 mM BCECF, AM *UltraPure grade* stock solution in high quality anhydrous DMSO.

a. Amount of BCECF, AM *UltraPure grade* to use: 1 mg
b. Desired concentration: 2 mM
c. In a suitable container mix 1 mg of BCECF, AM *UltraPure grade* with 618.28 µL of anhydrous DMSO.

3. Prepare a 2X working solution in HHBS with 10 µM BCECF, AM *UltraPure grade*[3], and 0.08% Pluronic® F-127.

a. Final in-well concentration of BCECF, AM *UltraPure grade*: 5 µM
b. Final in-well concentration of Pluronic® F-127: 0.04 %
c. In a suitable container mix 16 µL of BCECF, AM *UltraPure grade*, and 25.6 µL of 10% Pluronic® F-127. Next, add HHBS or a buffer of your choice until the volume is 3.2 mL.

*Note:For most cell lines we recommend the final concentration of BCECF, AM *UltraPure grade* be 2 to 5 µM.*
Note: Recommended final in well concentration of Pluronic F-127 is 0.02% to 0.04%.

4. Add 100 µL of the dye working solution into the desired wells already containing 100 µL of culture medium.

a. This step will dilute the dye working solution from 2X to 1X and adjust the final concentrations of each component to the following: 5 µM of BCECF, AM *UltraPure grade*, 0.04% Pluronic® F-127

5. Incubate the dye-loading plate[5].

a. Incubate the dye-loading plate in a cell incubator for 30-60 minutes.
b. Incubate the dye-loading plate at room temperature for 30 minutes.

6. Prepare an HHBS buffer (or a buffer of your choice).

a. In a suitable container add HHBS or a buffer of your choice until the volume is 4 mL.

7. Replace the dye working solution with the HHBS buffer or a buffer of your choice with 1.0 mM Probenecid.

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a. First, remove 200 µL of the dye working solution and culture medium from the desired wells.
b. To those same wells add back 200 µL of HHBS (or a buffer of your choice) with 1.0 mM Probenecid.

8. Run your assay.
a. Add desired treatment to your sample.
b. Run the experiment as Ex/Em = 503/528 nm.

Additional Information:

BCECF, AM *UltraPure grade* Specifications

Excitation:	503
Emission:	528
Molecular Weight:	808.69
Solvent:	DMSO
Extinction Coefficient:	N/A
K_d:	nM

1 M NaOH Recipe

1. Prepare 2 mL of distilled water in a suitable container.
2. Slowly add 100 mg of NaOH to the solution with mixing. *
3. Add distilled water until volume is 2.5 mL.
4. Store solution in plastic container at room temperature.

**This is an exothermic process, proper precautions and guidelines should be followed.*

10% Pluronic F-127 Recipe:

1. Dissolve 1 g of Pluronic® F-127 (Cat# 20050) in 10 mL of distilled water to make a 10% (w/v) stock solution.
2. Heat 10% Pluronic® F-127 stock solution for about 30 minutes at a temperature ranging from 40 to 50 °C.
3. Store excess 10% Pluronic® F-127 according to its storage specifications.

Storage Conditions

- It is recommened to prepare and use BCECF, AM *UltraPure grade* stock solution on the same day. However, if stock solutions need to be prepared in advanced we recommend storing the BCECF, AM *UltraPure grade* stock solution as aliquots in tightly sealed vials at -20°C, dessicated and protected from light. Under these conditions, AM esters should be stable for 3 months.
- 10% Pluronic F-127 stock solution must be stored at room temperature (DO NOT FREEZE) for up to 6 months.

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Notes

1. Volumes can be adjusted according to the need and volume of the experiment setups.
2. Pluronic® F-127 (PF-127) is a nonionic surfactant and relatively non-toxic to cells. PF-127 is commonly used with dye AM esters to improve their aqueous solubility.
3. The exact concentration of the indicator required for cell loading must be determined empirically.