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Protocol for Loading cAMP AM Into Live Cells

IMPORTANT DISCLAIMER: The following is a sample protocol for loading cAMP AM 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 cAMP AM. For assistance, use tools and calculators to determine the amount of component required for each part of the loading procedure.

Key parameters

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

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

☑ cAMP AM

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

☑ 100% DMSO

Optional

Step-by-step guide:

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1. Prepare an HHBS buffer.

a. For instructions on how to prepare a HHBS buffer, see our buffer recipe page

2. Prepare a to cAMP AM stock solution in high quality anhydrous DMSO.

a. Amount of cAMP AM to use: 1 mg
b. Desired concentration: 2 mM
c. In a suitable container mix 1 mg of cAMP AM with 1246.04 µL of anhydrous DMSO.

3. Prepare a 2X working solution in HHBS with 10 µM cAMP AM [2].

a. Final in-well concentration of cAMP AM: 5 µM
b. In a suitable container mix 16 µL of cAMP AM. 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 cAMP AM be 2 to 5 µM.

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 cAMP AM

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

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

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.

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 = / nm.

Additional Information:

cAMP AM Specifications

Excitation:	
Emission:	
Molecular Weight:	401.27
Solvent:	DMSO
Extinction Coefficient:	N/A
K_d:	nM

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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.*

Storage Conditions

- It is recommened to prepare and use cAMP AM stock solution on the same day. However, if stock solutions need to be prepared in advanced we recommend storing the cAMP AM 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.

Notes

1. Volumes can be adjusted according to the need and volume of the experiment setups.
2. The exact concentration of the indicator required for cell loading must be determined empirically.
3.