Fluorescent Dye AM Esters

Introduction

Fluorescent dye AM esters are hydrophobic compounds that easily permeates intact live cells, and are widely used for loading a variety of polar fluorescent probes into live cells non-invasively. The non-polar AM esters readily cross live cell membranes, and rapidly hydrolyzed by cellular esterases inside live cells. The hydrolysis of the esterified groups is essential for analyzing a variety of cellular functions. In some cases (e.g., calcein AM), the AM ester is colorless and non-fluorescent until hydrolyzed.

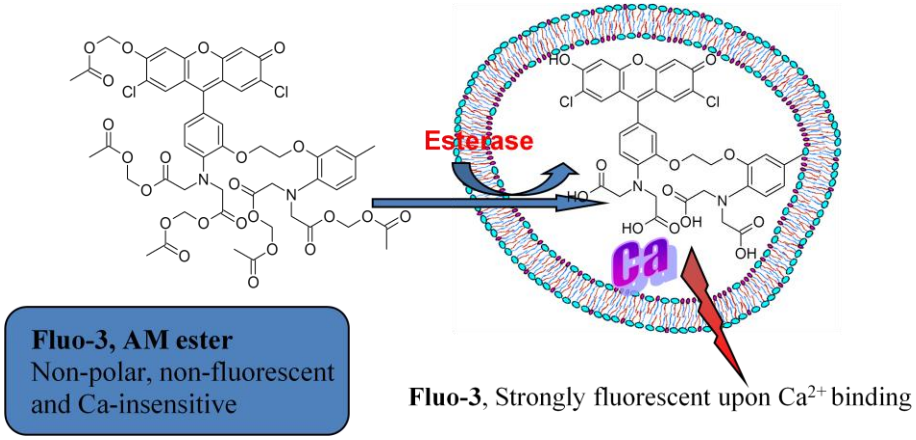

Figure 1. The principle of Fluo-3 AM is loaded into live cell for intracellular Ca^{2+} detection.

Storage Conditions

Store at –20 ^{0}C, protected from light. Expiration date is 6 months from the date of receipt.

Prepare the AM Ester Stock Solutions

a) Prepare a 1 to 10 mM stock solution of AM esters just before use in high quality, anhydrous dimethylsulfoxide (DMSO). DMSO stock solutions should be stored well sealed, kept desiccated at - 20 °C and protected from light. Under these conditions, AM esters should be stable for several months.
Note: AM esters are susceptible to hydrolysis, particularly in solution. So keep the AM ester stock solutions as concentrated as possible for optimal results. Minimum water content of DMSO (ideally ≤ 0.1%) should be added into the loading solution.

b) The nonionic detergent Pluronic® F-127 is sometimes used to increase the aqueous solubility of AM esters.
Note: An equal volume of 20% Pluronic® F-127 solution can be added to DMSO stock solutions before diluting into the loading buffer. The final Pluronic® F-127 concentration is about 0.02%. A variety of Pluronic® F-127 solutions can be purchased from AAT Bioquest. The long-term storage of AM esters in the presence of Pluronic® F-127 is not recommended.

Loading Cells with AM Esters

Following is our recommended protocol for loading AM esters into live cells. This protocol only provides a guideline, and should be modified according to your specific needs.

a) On the day of the experiment, either dissolve AM esters in DMSO or thaw an aliquot of the indicator stock solutions to room temperature. Prepare a working solution of 1 to 10 µM (higher concentrations of weakly fluorescent indicators may be required) in the buffer of your choice (such as Hanks and Hepes buffer). For most of cell lines, AM esters (4-5 µM) are recommended. The exact concentration of indicators required for cell loading must be determined empirically. To avoid any artifacts caused by overloading and potential dye toxicity, it is recommended to use the minimal dye concentration that can generate sufficient signal strength.

b) If the cells containing the organic anion-transports, probenecid (1-2.5 mM) or sulfinpyrazone (0.1-0.25 mM) may be added to the cell medium to reduce leakage of the de-esterified indicators. Incubate cells with the AM esters for 20 minutes to one hour (Longer incubation time is required for weakly fluorescent indicators) at room temperature or 37 °C.
Note: Decreasing the loading temperature might reduce the compartmentalization of the indicators.

c) Wash cells in dye-free buffers (containing an anion transporter inhibitor, if applicable) to remove excess probes.

d) Measure fluorescence intensity at desired Ex/Em wavelengths.

AM Ester Products

The following tables summarized current AM ester products from AAT Bioquest.

Table 1. UV-Excitable Calcium Indicators

Ca^{2+} Indicator	**Product Numbers**	**Excitation**	**Emission**	**MW**
Calcein blue, AM	21007	360 nm	445 nm	465.41
CytoCalcein Violet 450	22012	408 nm	460 nm	~400
Fura-2, AM	21020, 21021, 21022, 21023,	340/380 nm	510 nm	1001.86
Indo-1, AM	21030, 21032, 21033, 21036,	355 nm	400/475 nm	1009.91
Quin-2, AM	21050	346 nm	475 nm	829.76

Table 2. Visible Light-Excitable Calcium Indicators

Ca^{2+} Indicator	**Product Numbers**	**Excitation**	**Emission**	**MW**
Calcein, AM	22002, 22003, 22004, 22005	490 nm	515 nm	994.86
Fluo-3, AM	21010, 21011, 21012, 21013, 21014	506 nm	526 nm	1129.85
Quest Fluo™-8™, AM	21080, 21081, 21082, 21083	490 nm	514 nm	~1000
Quest Fluo™-8H™, AM	21090, 21091	490 nm	514 nm	~1100
Quest Fluo-8L™, AM	21096, 21097	490 nm	514 nm	~1100
Quest Rhod-4™, AM	21120, 21121, 21122, 21123	530 nm	555 nm	~1000
Rhod-2, AM	21060, 21062, 21063, 21064, 21065	549 nm	578 nm	1123.96
Rhod-5N, AM	21070	551 nm	577 nm	1154.92

Table 3. Non-Fluorescent Calcium Detection AM esters

Ca^{2+} Indicator	**Product Numbers**	**MW**
BAPTA, AM	21001, 21002	764.68
EGTA AM	21005, 21006	668.6

Table 4. Other Ion Fluorescent Indicators

AM Ester	**Product Numbers**	**Excitation**	**Emission**	**MW**
BCECF, AM	21202, 21203, 21204	505 nm	520 nm	808.69

References

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