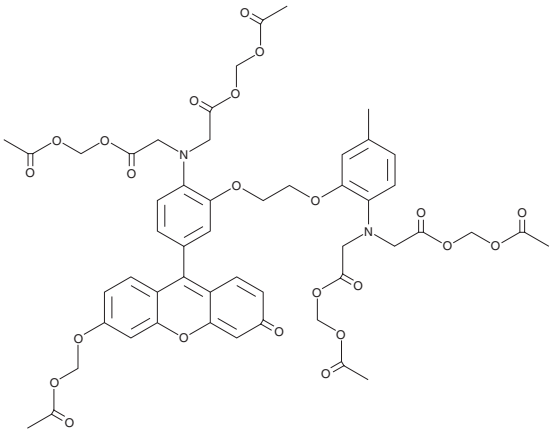
PRODUCT INFORMATION

Fluo-2 AM

Item No. 35758



CAS Registry No.: 1070771-36-6
Formal Name: N-[2-[(acetyloxy)methoxy]-2-oxoethyl]-N-[4-[6-[(acetyloxy)methoxy]-3-oxo-3H-xanthen-9-yl]-2-[2-[2-[*bis*[2-[(acetyloxy)methoxy]-2-oxoethyl]amino]-5-methylphenoxy]ethoxy]phenyl]-glycine (acetyloxy)methyl ester
Synonyms: Fluo-2 Acetoxymethyl ester, Fluo-2 HA, Fluo-2 High Affinity
MF: $C_{51}H_{52}N_2O_{23}$
FW: 1,061.0
Purity: ≥95%
UV/Vis.: λ_{max}: 254 nm
Ex./Em. Max: 490/515 nm
Supplied as: A solid
Storage: -20°C
Stability: ≥4 years
Special Conditions: Protect from light and moisture

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Description

Fluo-2 AM is a cell-permeable fluorescent calcium indicator.[1,2] It has been used to measure the activity of the neuropeptide Y_4 receptor, as well as to detect intracellular calcium in tetrandine-stimulated primary rabbit corpus cavernosum smooth muscle cells. It binds to calcium (K_d = 290 nM) and displays excitation/emission maxima of 490/515 nm, respectively. Fluo-2 AM is also available in a cell-impermeable form (Item No. 35764).

Assay Protocol

Note: Allow all reagents to warm to room temperature before proceeding.

1. Add 10 ml of assay buffer to a 15 or 50 ml conical tube.

Note 1: HEPES-buffered Hank's balanced salt solution (HBSS), pH 7.2-7.4, is recommended, although other buffers can be used.

2. Add 100 µl of a 100X Pluronic™ F-127 solution (1-50% w/v) to the conical tube*. Pluronic™ F-127 is a biocompatible surfactant used to ensure equitable dye distribution and cellular loading.
    a. Optional: Add 100 µl of 2 mM probenecid stock solution to the conical tube. Probenecid (Item No. 14981) is an anion transport inhibitor used to improve intracellular dye retention. Use of probenecid is recommended, but not required, for all cell types and dyes.

**Final working concentration of Pluronic™ F-127 should be between 0.01 and 0.5% w/v. User should optimize the concentration of Pluronic™ F-127 to suit experimental requirements.*

Note 2: Probenecid is an inhibitor or agonist of multiple ion channels and may have undesirable cellular effects that could affect dye performance.

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3. Vortex conical tube briefly to mix.
4. Dissolve Fluo-2 AM in 25 µl of DMSO and vortex dye tube briefly to mix.
5. Centrifuge dye tube briefly to collect all contents at the tube bottom.
6. Add entire contents of dye tube to the conical tube containing the assay buffer solution to make the dye loading solution.
7. Vortex conical tube briefly to mix.

Note 3: The dye loading solution should be used within two hours for best results.

8. Remove cell culture medium and add dye loading solution. Recommended volumes are:
    a. 35 mm dish or 6-well plate: 1.5 ml/dish or well
    b. 96-well plate: 100 µl/well
    c. 384-well plate: 20 µl/well

Note 4: To prevent cell detachment or if using suspension cells, the dye loading solution can be added directly to the media-containing wells. User must double the component concentrations to achieve the same final concentration of all reagents.

9. Incubate cells with the dye loading solution at 37°C for 60 minutes.
10. Read fluorescence using a plate reader at excitation and emission wavelengths of 490 and 515 nm, respectively.
    Or
    Image using a fluorescence microscope with filters for GFP or fluorescein.

References

1. Sliwoski, G., Schubert, M., Stichel, J., *et al*. Discovery of small-molecule modulators of the human Y_4 receptor. *PLoS One* **11(6)**, e0157146 (2016).
2. Liu, J.-H., Chen, J., Wang, T., *et al*. Effects of tetrandrine on cytosolic free calcium concentration in corpus cavernosum smooth muscle cells of rabbits. *Asian J. Androl*. **8(4)**, 405-409 (2006).

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