

Calcium protocols

(from www.dartmouth.edu/~celllab/confocal/calcium.html, reviewed and commented by David Pflugh)

Indo-1-AM: purchased from Molecular Probes as 50 µg in each of 20 vials (1-800-438-2209 Cat #I-1223, approx. price \$128) mwt 1010. Store in freezer.

When needed, add 50 µl of DMSO to the 50 µg in one vial. Dissolve well → 1 mM stock solution.

For cell incubation, dilute 5 µl of the 1 mM stock into 1 ml of serum free medium → 5 µM final concentration for incubation. Incubate cells (at about 10⁷ml) in this 5 µM Indo for 30 min at 37 degrees.

Wash cells well in calcium-containing medium; then keep cells at room temperature before use.

Cell will probably be OK for several hours if kept at room temperature (Indo leaks out rapidly at 37 degrees). Or sulfinpyrazone (Sigma) can be added at the same time as the Indo-1 to the cells at 0.1 to 0.25 mM to reduce the leakage of the de-esterified Indo-1.

The 1 mM stock solution of Indo in DMSO can be kept in the freezer and used again for several months.

Pluronic detergent: is given away free of charge by Molecular Probes. Can be useful for loading difficult cells, e.g. CHO cells or others which have strong efflux pumps. Make up a 20% stock solution of pluronic in DMSO. If cells don't load well, add 1 µl of this detergent stock to 1 ml of cell suspension being loaded with Indo (as above) to give a final 0.02%

detergent solution.

Ionomycin, free acid, purchased as 1 mg from Calbiochem. mwt 709.

Add 357 μ l of DMSO to 1 mg of ionomycin \rightarrow 4 mM stock solution.
Store in freezer.

For ACAS:

When needed, add 5 μ l of inomycin stock to 5 ml of reaction medium --
4 μ M solution.

Dilute 1:2 by addition to cells in chamber on ACAS \rightarrow 2 μ M final
concentration.

For Flow:

When needed, add 5 μ l of ionomycin stock to 45 μ l of reaction medium
 \rightarrow 0.4 μ M solution. Dilute 1:200 by addition to flow sample \rightarrow 2 μ M
final concentration.

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