

FDA Vital Cell Stain

Use < 0.5 mg/ml

1. Prepare cells in standard way
2. Lyse RBC's - see Hemolyte Gey's treatment (not necessary)
3. Resuspend cells to 10^7 /ml (not critical-can be as low as 10^6 /ml)
4. Add 2λ of FDA at 5 mg/ml in acetone per 1 ml cells at 10^7 /ml.
5. Incubate at R.T. for \sim 20 min. away from light.
6. Wash 3X in medium-resuspend to appropriate concentration (live cells will exhibit fluorochromasia)

Source - Fluorescein Diacetate (FDA)

powder form purchased from Mann Research.

FDA is a non-fluorescing derivative of fluorescein, which rapidly penetrates cells. Once the label is inside the cell, it is hydrolyzed by esterases to produce fluorescein which is not readily extruded and so accumulates inside living cells causing them to fluoresce brightly under blue light. Cytotoxicity of leucocytes by action of antibody and complement results in the rapid leaking out of the fluorescein.

Store FDA solution in dark in freezer and in a tightly stoppered bottle.

Kolman, B., and Papameter, D., 1966, *The use of a vital cell stain*
in studying the effects of antibodies on cells. *Journal of Immunology*,
99: 134-138.
PNAS, U.S.A. 55: 134.