

SOP-P006

Preparation of 20 mM DCFH-DA

Objective: To prepare a solution of 2',7'-dichlorofluorescein diacetate (DCFH-DA) in absolute ethanol for staining cells for the detection of hydrogen peroxide release in functional assays using flow or image cytometry

Procedure:

1. Weigh out a quantity of DCFH-DA between 2 and 9 mg on a square of glassine paper.
2. Transfer the dye immediately to a 12 x 75 mm foil-wrapped test tube with stopper.
- 3a. Calculate the volume of ethanol required in ml by dividing the actual weight in mg of the dye by 9.76 (20mM DCFH-DA is 9.76mg/ml).
- 3b. Pipet the calculated volume of absolute (anhydrous) ethanol into the tube.
4. Stopper the tube and mix well. Check to see that the dye has dissolved.
5. Label the tube with content information, date, and your initials. Keep the dye solution refrigerated at 0-5°C and discard unused portion at the end of the day.

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Verified by: _____ **Date:** _____
Print Name Sign Name