

Fluorescent dye stock solutions for Flat Field Correction

Purpose

This protocol describes how to prepare stocks of fluorescent dye solutions from solid powder and the further dilution required to make the working Flat Field solutions.

Related SOPs

The following SOPs are used in conjunction with this protocol.

1. Cell plating for imaging v1.0
2. Microscopy Pipeline Workflow – Image Acquisition v1.0

Reagents

General method for the reconstitution of fluorescent dye solution

Reconstitution of Coumarin 102 (catalog# 41267-76-9, 546151-100 mg from Sigma-Aldrich, MW: 256.31g/mol).

1. Calculate the required amount of DMSO to reconstitute the Coumarin solid powder at a 100mM stock concentration:

DMSO volume [mL]:

$$\frac{0.1 \text{ g}}{256.31 \frac{\text{g}}{\text{mol}}} * \frac{1}{0.1 \frac{\text{mol}}{\text{L}}} * \frac{10^3 \text{ mL}}{1 \text{ L}} = 3.9 \text{ mL DMSO}$$

to make a 100 mM stock from 100 mg of solid Coumarin 102 dye powder.

2. Vortex stock solution at highest speed setting for 5 min. Aliquot stock solution into 500 μL aliquots and store at -20°C when not in use. Limit the number of freeze thaw cycles to less than 10.

Working solution of Coumarin 102 preparation:

1. Dilute coumarin stock solution 1:50 into DMSO to obtain a final dye concentration solution of 2mM, (i.e for 1 mL add 20 μL of coumarin stock solution to 980 μL DMSO, and mix well).
2. Filter final solution with a syringe filter (0.20 μm Supor membrane, Acrodisc® Syringe Filters with Supor® Membrane, 0.8/0.2 μm , 32 mm, catalog #4652, PALL Life Sciences) into a 15mL conical tube. **Make fresh daily.**

Reconstitution of Fluorescein (catalog# 2321-07-5, 32615-25g from Sigma-Aldrich, MW: 332.31).

1. Calculate the required amount of PBS needed to reconstitute 0.04 g of Fluorescein solid powder to provide a stock solution at a concentration of 2.4 mM.

PBS volume [mL]:

$$\frac{0.04 \text{ g}}{332.31 \frac{\text{g}}{\text{mol}}} * \frac{1}{0.0024 \frac{\text{mol}}{\text{L}}} * \frac{10^3 \text{ mL}}{1 \text{ L}} = 50 \text{ mL PBS}$$

required to make a 2.4 mM stock solution from 40 mg of solid Fluorescein dye powder.

2. Vortex stock solution at high speed for 5 min. Store stock solution into 50 mL Falcon tube at 4°C when not in use. Make appropriate volume aliquots to limit the number of freeze thaw cycles to less than 10, (1mL aliquots if kept at -20°C when not in use).

Working solution of Fluorescein 102 preparation:

1. Dilute fluorescein stock solution 1:10 into DMSO to obtain a final concentration of 240µM (i.e. for 1mL, add 100 µL (2.4 mM) of fluorescein stock solution to 900 µL of DMSO and mix well).
2. Filter final solution with a syringe filter (0.20µm Supor membrane, Acrodisc® Syringe Filters with Supor® Membrane, 0.8/0.2 µm, 32 mm, catalog# 4652, PALL Life Sciences) into a 15mL conical tube. **Make fresh daily.**

Reconstitution of Acid Blue 9 (catalog# 3844-45-9 CI-42090-25g from TCI, MW 792.84)

1. Calculate the required amount of DMSO to reconstitute the Acid Blue 9 fluorophore solid powder at a 10 mM stock concentration.

DMSO required volume [mL]:

$$\frac{0.792 \text{ g}}{792.84 \frac{\text{g}}{\text{mol}}} * \frac{1}{0.01 \frac{\text{mol}}{\text{L}}} * \frac{10^3 \text{ mL}}{1 \text{ L}} = 99 \text{ mL DMSO}$$

required to make a 10 mM stock from 0.8 g of solid Acid Blue 9 dye powder.

2. Vortex stock solution at high speed for 5min. Store stock solution in 1mL aliquots at -20°C when not in use. Make appropriate volume aliquots to limit the number of freeze thaw cycles to less than 10.

Working solution of Acid Blue 9 preparation:

1. Dilute the Acid Blue 9/DMSO stock solution 1:5 into DMSO to obtain a final dye concentration solution of 2 mM (i.e. for 1mL, add 200 μ L of acid blue 9 stock solution to 800 μ L DMSO and mix well).
2. Filter final solution with a syringe filter (0.20 μ m Supor membrane Acrodisc® Syringe Filters with Supor® Membrane, 0.8/0.2 μ m, 32 mm, cat#4652, PALL Life Sciences) into a 15mL conical tube. **Make fresh daily.**

Important Notes: Always vortex stock solutions before performing dilution for flat fielding samples. The final solution should also be vortexed before addition to imaging plate or glass slide to ensure homogeneity. The concentration of the diluted dye solutions should be adjusted to achieve minimum pixel intensity of 1000 grey level for each channel. For best results try to use the same laser power used to image the cells.

Preparation on the day of imaging

Add 150 μ L of the final dye solution concentration (described above) to wells D9, E9, and F9 of a Pipeline 96-well plate prior to imaging as follow:

	4	5	6	7	8	9
C	AICS0	AICS0	AICS0	AICS0	AICS0	TL (PR-mTESR)
D	AICS12cl10 5	AICS12cl10 5	AICS12cl10 5	AICS12cl10 5	AICS12cl10 5	Acid blue
E	exp line	exp line	exp line	exp line	exp line	Coumarin
F	exp line	exp line	exp line	exp line	exp line	Fluorescein
G						Microspheres

Proceed with imaging according to section: *Imaging of Optical controls* of SOP: Microscopy Pipeline Workflow – Image Acquisition.