

Fixing Cell Pellets for Flow Cytometry V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bixakfie

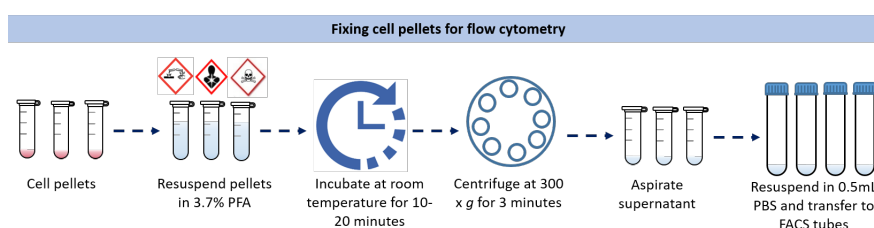
Cellular Generation and Phenotyping

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ABSTRACT

This protocol is designed to outline the process of fixing cell pellets in 1.5ml tubes. It has been developed within the Cellular Generation and Phenotyping Group at the Wellcome Sanger Institute.

Process diagram:



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MATERIALS

NAME	CATALOG #	VENDOR
DPBS	14190	Invitrogen - Thermo Fisher

NAME	CATALOG #	VENDOR
Falcon Round bottomed 5ml tube with cell strainer lid	734-0001	VWR international Ltd
37% Formaldehyde	A0823	AppliChem

MATERIALS TEXT

Equipment

- Chemical Fume Hood
- Microfuge
- Rainin Single Channel Electronic Pipette LTS E4 1000XLS+
- Rainin 1200ul tips
- Pipetboy
- Stripettes

SAFETY WARNINGS

Please refer to the manufacturer's documentation and material safety data sheets (MSDS) for the products you are using when following this protocol.



Formaldehyde: Toxic if swallowed, in contact with skin or if inhaled. Causes severe skin burns and eye damage. May cause an allergic skin reaction. May cause respiratory irritation. Suspected of causes genetic defects. May cause cancer. Causes damage to organs.

BEFORE STARTING

This protocol must be carried out in a chemical fume hood.

Fixing

- 1 In a fume hood, prepare a solution of 3.7% formaldehyde in PBS



Chemical safety: Formaldehyde 37% and 3.7% must be prepared and used only in the chemical fume hood, using chemical resistant gloves. Waste must be kept in the fume hood and disposed of appropriately.

- 2 In the fume hood, tap the tube to loosen the pellet and resuspend each pellet in **500 µl** of 3.7% formaldehyde. Mix well by pipetting up and down, ideally using a Rainin 1ml electronic pipette to obtain a single cell suspension and incubate at room temperature to fix for 10-20 minutes.
- 3 Centrifuge cells at **300 x g 00:03:00** using a minifuge inside the fume hood.
- 4 Carefully remove the supernatant (dispose of waste formaldehyde appropriately) and resuspend the cell pellet in **500 µl** PBS and transfer to a 5ml BD Falcon Round Bottom Polystyrene Tube by pipetting through its filtered cap.



At this stage, the fixed cell suspension can either be stored at **4 °C** overnight or analysed using flow cytometry immediately.

