Fixing cell pellets V.1

Verity Goodwin¹, Emily Souster¹, Mathew Garnett¹, Fiona Behan¹, Charlotte Beaver¹, Rizwan Ansari¹, Adam Jackson¹
¹Wellcome Sanger Institute

Citation: Verity Goodwin, Emily Souster, Mathew Garnett, Fiona Behan, Charlotte Beaver, Rizwan Ansari, Adam Jackson (07/22/2020). Fixing cell pellets. protocols.io
https://dx.doi.org/10.17504/protocols.io.bg2fjybn

Cellular Generation and Phenotyping

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:

- Fixing cell pellets for flow cytometry

   Cell pellets → Resuspend pellets in 3.7% PFA → Incubate at room temperature for 10-20 minutes → Centrifuge at 300 x g for 5 minutes → Aspirate supernatant → Resuspend in 5.5ml PBS and transfer to FACS tubes

DOI
dx.doi.org/10.17504/protocols.io.bg2fjybn

PROTOCOL CITATION
Verity Goodwin, Emily Souster, Mathew Garnett, Fiona Behan, Charlotte Beaver, Rizwan Ansari, Adam Jackson 2020. Fixing cell pellets. protocols.io
https://dx.doi.org/10.17504/protocols.io.bg2fjybn

LICENSE
This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

CREATED
Jun 01, 2020

LAST MODIFIED
Jul 22, 2020

PROTOCOL INTEGER ID
37671
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 for 3 minutes 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.
At this stage, the fixed cell suspension can either be stored at 4 °C overnight or analysed using flow cytometry immediately.