Preparing primary T cells for fluorescence microscopy

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MATERIALS

- PBS Invitrogen - Thermo Fisher
- BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit BD Biosciences Catalog #554714
- Pierce™ 16% Formaldehyde (w/v) Methanol-free Thermo Fisher Scientific Catalog #28906
- Alexa Fluor™ 488 Phalloidin Contributed by users Catalog #A12379
- ProLong™ Glass Antifade Mountant with NucBlue™ Stain Thermo Fisher Scientific Catalog #P36983
- Cytology Funnels for Shandon CytoSpin™ Centrifuge Biomedical Polymers VWR international Ltd Catalog #80094-254

MANUSCRIPT CITATION:

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Protocol status: Working
We use this protocol and it's working

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Cell Culture

1. Culture primary T cells using standard tissue culture techniques.

Fixation, permeabilization, and staining

2. Collect 200K to 1 million cells and sping them at 300g for 00:05:00.

3. Discard the supernatant, resuspend the cells in 1 mL PBS, spin them at 300g for 00:05:00, and discard the supernatant.

4. Fix the cells:
   - If the cells are too clumpy and sticking to each other, first resuspend them in 750 µL PBS and mix them thoroughly until there are no clumps and add 250 µL of 16% formaldehyde on top.
   - If there are no cell clumps, simply resuspend cells in 1 mL of Cytofix solution.

5. Incubate the tubes at 4 ºC for 00:30:00 on a nutating mixer, e.g.
6  Spin the cells at 300g for \(00:05:00\), discard the supernatant, resuspend them in \(1\ mL\) of 1X CytoPerm Wash Buffer.

7  Optionally, add your stains, dyes (e.g. phalloidin stain) into the permeabilization buffer.

8  Cover the tubes with aluminum foil and incubate at \(25^\circ \text{C (Room temperature)}\) for \(00:30:00\) or for the specific duration required for the stain on a nutating mixer, e.g. ...
Spin the cells at 300g for 00:05:00, discard the supernatant, resuspend them in 1 mL of PBS.

Spin the cells at 300g for 00:05:00, discard the supernatant, resuspend them in 200 µL of PBS.

Cytospin 200 µL of the stained cell suspension onto a glass slide or sample cover slip at 300-500g for 00:05:00.

Mount the sample in between a glass slide and cover slip using one drop (at least 10 µL) of ProLong Anti-fade reagent and keep the slide within a dark container for at least 12:00:00.