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 We use this protocol and it's working

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Preparing primary T cells for fluorescence microscopy

In 1 collection

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MATERIALS

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⊗ 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**

Cell Culture

- 1 Culture primary T cells using standard tissue culture techniques

Fixation, permeabilization, and staining

- 2 Collect 200K to 1 million cells and spin 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 μ L PBS and mix them thoroughly until there are no clumps and add 250 μ L of 16% formaldehyde on top.

If there are no cell clumps, simply resuspend cells in 1 mL of Cytifix solution.

- 5 Incubate the tubes at 4 °C for 00:30:00 on a nutating mixer, e.g.

Equipment

Fisherbrand™ Nutrating Mixers - Variable Speed

NAME

Mixer/Shaker

TYPE

Fisherbrand

BRAND

88-861-043

SKU

<https://www.fishersci.com/shop/products/nutating-mixers-variable-speed/p-6707018#?keyword=nutating+mixer> LINK

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 aliminum foil and incubate at 25 °C (Room temperature) for 00:30:00 or for the specific duration required for the stain on a nutating mixer, e.g.

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9 Spin the cells at 300g for 00:05:00 , discard the supernatant, resuspend them in 1 mL of PBS

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

Slide preparation

- 11 Cytospin 200 μL of the stained cell suspension onto a glass slide or sample cover slip at 300-500g for 00:05:00
- 12 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