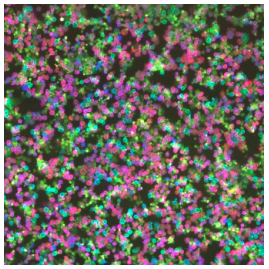
Nov 07, 2018

Preparing primary T cells for fluorescence microscopy



In 1 collection

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Bulent Arman Aksoy[1], Pinar Aksoy[1], Jeff Hammerbacher[1]

[1]Medical University of South Carolina

Hammer Lab
Tech. support phone: **+18437924527** email: **arman@hammerlab.org**

Bulent Arman Aksoy
Medical University of South Carolina

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Manuscript citation:

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

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Materials

MATERIALS

- PBS **Invitrogen - Thermo Fisher**
- BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit **Becton Dickinson (BD)** **Catalog #**554714
- Pierce™ 16% Formaldehyde (w/v) Methanol-free **Thermo Fisher Scientific** **Catalog #**28906
- Alexa Fluor™ 488 Phalloidin **Catalog #**A12379
- ProLong™ Glass Antifade Mountant with NucBlue™ Stain **Thermo Fisher Scientific** **Catalog #**P36983
- Cytology Funnels for Shandon CytoSpin™ Centrifuge Biomedical Polymers **VWR International (Avantor)** **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 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 thouroughly until there are no clumps and add 250 µL of 16% formaldhyde on top.

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

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

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 uL) of ProLong Anti-fade reagent and keep the slide within a dark container for at least 12:00:00