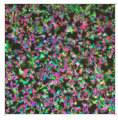

Preparing primary T cells for fluorescence microscopy

Bulent Arman Aksoy[1], PINAR AKSOY[1], Jeff Hammerbacher[1]
[1]Medical University of South Carolina

dx.doi.org/10.17504/protocols.io.vede3a6

Bulent Arman Aksoy
Medical University of South Ca...

TAGS

microscopy

dapi

Show tags

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

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

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 Mixer/Shaker
Fisherbrand 88-861-043

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 Mixer/Shaker
Fisherbrand 88-861-043

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

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited