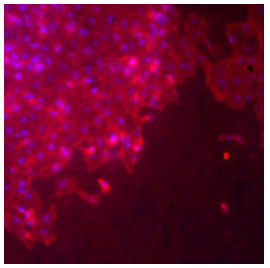
Feb 18, 2019 Version 3

Mammalian Cell Staining V.3



DOI
dx.doi.org/10.17504/protocols.io.x95fr86

Kenneth Schackart[1], Kattika Kaarj[1]

[1]University of Arizona

481b Laboratory

Kenneth Schackart
University of Arizona

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.x95fr86

Protocol Citation: Kenneth Schackart, Kattika Kaarj 2019. Mammalian Cell Staining. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.x95fr86**

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

Created: February 18, 2019

Last Modified: February 18, 2019

Protocol Integer ID: 20509

Keywords: mammalian cell, cell, actin filament, focal adhesion site, well plate

Abstract

This protocol details how to stain mammalian cells cultured on a 96-well plate. Actin filaments, focal adhesion sites (as indicated by the presence of vinculin), and nuclei will be stained.

Materials

- 4% Paraformaldehyde solution
- 0.1% Triton X-100 solution in PBS
- Blocking buffer (PBS + 1% bovine serum albumin)
- Washing buffer (PBS + 0.05% Tween-20)
- Anti-vinculin solution (1:500 in blocking buffer)
- TRITC-conjugated phalloidin and FITC-conjugated antivinculin secondary antibody solution (1:1:248, TRITC:FITC:blocking buffer) referred to as FITC:TRITC
- DAPI solution (1:249 DAPI:blocking buffer)
- Phosphate buffered saline (PBS)

Troubleshooting

Fix the cells

1 Remove cell culture media.

2 Add 100 µL of [M] 4 % volume paraformaldehyde solution.

3 Incubate for 00:05:00 .

Perforate cell membrane

4 Remove paraformaldehyde solution.

5 Wash twice with 100 µL washing buffer.

> Note
>
> Washing buffer is PBS with the detergent Tween-20.

6 Add 100 µL of [M] 0.1 % volume Triton X-100.

7 Incubate for 00:05:00 .

Block unspecific binding

8 Remove Triton X-100.

9 Wash twice with 100 µL washing buffer.

10 Add 100 µL blocking buffer.

Note

Blocking buffer is PBS with BSA (bovine serum albumin) and is used to prevent unspecific binding.

11 Incubate for 00:10:00 .

Bind anti-vinculin to vinculin

12 Remove blocking buffer.

Bind anti-vinculin to vinculin

13 Wash twice with washing buffer.

Bind anti-vinculin to vinculin

14 Add 250 µL of Anti-Vinculin and blocking buffer mixture.

Bind anti-vinculin to vinculin

15 Incubate for 00:20:00 .

Stain actin filaments and focal adhesion sites

16 Remove anti-vinculin blocking buffer mixture.

Stain actin filaments and focal adhesion sites

17 Wash twice.

18 Add 100 µL FITC:TRITC solution, cover in foil.

19 Incubate for 00:30:00 .

Stain nuclei

20 Remove stains.

21 Add 100 µL of DAPI solution, cover in foil.

22 Incubate for 00:05:00

23 Remove DAPI solution.

24 Add 100 µL PBS.

Image

25 Image your cells using UV, Blue, and Green excitation.