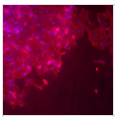

Mammalian Cell Staining

Version 3

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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.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS TEXT

- 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)

Fix the cells

1 Remove cell culture media.

2 Add **100 µl** of **[M]4 Volume Percent** paraformaldehyde solution.

3 Incubate for **00:05:00** .

Perforate cell membrane

4 Remove paraformaldehyde solution.

5 Wash twice with **100 µl** washing buffer.

Washing buffer is PBS with the detergent Tween-20.

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6 Add **100 µl** of **[M]0.1 Volume Percent** 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.

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.

13 Wash twice with washing buffer.

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

15 Incubate for **00:20:00** .

Stain actin filaments and focal adhesion sites

16 Remove anti-vinculin blocking buffer mixture.

17 Wash twice.

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

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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.

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