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 We use this protocol and it's working

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Mammalian Cell Staining V.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.

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)

Fix the cells

- 1 Remove cell culture media.
- 2 Add 100 μ L of $[M]$ 4 % volume paraformaldehyde solution.
- 3 Incubate for 00:05:00 .

Perforate cell membrane

- 4 Remove paraformaldehyde solution.
- 5 Wash twice with 100 μ L washing buffer.

Note

Washing buffer is PBS with the detergent Tween-20.

- 6 Add 100 μ L 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.

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 μ L FITC:TRITC solution, cover in foil.

19 Incubate for 00:30:00 .

Stain nuclei

20 Remove stains.

21 Add 100 μ L of DAPI solution, cover in foil.

22 Incubate for 00:05:00

23 Remove DAPI solution.

24 Add 100 μ L PBS.

Image

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