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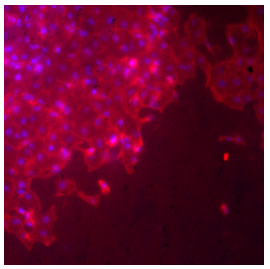
Mammalian Cell Staining V.1



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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 vinculin), and nuclei will be stained.

Materials

- 4% Paraformaldehyde solution
- 0.1% Triton X-100
- Blocking buffer
- Anti-vinculin with blocking buffer
- TRITC and FITC-conjugated secondary antibody solution
- DAPI solution
- Phosphate buffered saline (PBS)
- Dulbecco's phosphate buffered saline (DPBS)

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 DPBS

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 DPBS

10 Add 100 µL blocking buffer

11 Incubate for 00:10:00

Stain for focal adhesion sites and actin filaments

12 Remove blocking buffer

13 Wash twice with DPBS

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

15 Incubate for 00:20:00

16 Add 100 µL FITC-conjugated secondary antibody and TRITC

17 Incubate for 00:30:00

Stain nucleus

18 Remove stains

19 Add 100 µL of PBS