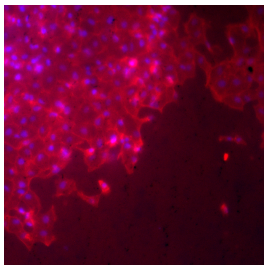
Feb 05, 2019 Version 2

Mammalian Cell Staining V.2



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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
- NucBlue™ LiveReady Probes™ Reagent 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 NucBlue™ solution.