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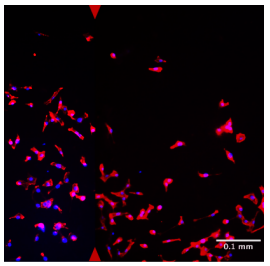
# Mammalian Cell Staining



Forked from Mammalian Cell Staining

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Kenneth Schackart[1]

[1]University of Arizona

Yoon Lab

Kenneth Schackart
University of Arizona



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

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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.

## Guidelines

- Gloves must be worn at all times.
- Perform all tasks within biosafety cabinet.
- Anything entering biosafety cabinet must be generously sprayed with 70% ethanol (even you).
- When finished, wipe biosafety cabinet with 70% ethanol, and UV for at least 15 minutes.

## Materials

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

## Before start

- Warm DPBS in 37 °C water bath.
- Wash waste beaker with soap and warm water, then dry with paper towel.
- Expose waste beaker to UV for at least 00:15:00 .

## Fix the cells

1 Remove cell culture media.

> Note
>
> You may wash twice with DPBS at this point.

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

3 Incubate for 00:15:00 .

## Perforate cell membrane

4 Remove paraformaldehyde solution.

5 Wash twice with 100 µL washing buffer.

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 washing buffer.

10 Add 100 µL blocking buffer.

11 Incubate for 00:30:00 .

## Bind anti-vinculin to vinculin

12 Remove blocking buffer.

13 Wash twice with washing buffer.

14 Add 100 µL of Anti-Vinculin and blocking buffer mixture.

15 Incubate for 01:00:00 .

> Note
>
> Increasing this duration can greatly increase FITC-conjugated secondary antibody binding. It is acceptable to place covered plate in refrigerator overnight. While waiting on this incubation, it is a good time to prepare the combined dye solution.

## Stain actin filaments and focal adhesion sites

16 Remove anti-vinculin blocking buffer mixture.

17 Wash twice.

18 Add 100 µL FITC:TRITC solution

19 Incubate for 00:30:00 .

## Stain nucleus

20 Remove stains.

21 Wash twice with washing buffer.

22 Add 100 µL of DAPI solution

23 Incubate for 00:05:00

24 Remove DAPI.

25 Add 100 µL PBS to prevent dessication of cells during imaging/storage.