Mammalian Cell Staining V.3

Kenneth Schackart¹, Kattika Kaarj¹

¹University of Arizona

481b Laboratory

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 µL** of **4% volume** paraformaldehyde solution.

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

**Perforate cell membrane**

4. Remove paraformaldehyde solution.

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

   **Note**
   
   Washing buffer is PBS with the detergent Tween-20.

6. Add **100 µL** of **0.1% volume** Triton X-100.
7 Incubate for 00:05:00.

8 **Block unspecific binding**

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 µL FITC:TRITC solution, cover in foil.

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.

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