

Cell Staining Protocol

Background:

With this protocol we will visualize cell structure and determine which patient cells over-express mER by staining:

- Membrane-bound Estrogen Receptor (mER)
- Actin (cytoskeleton)
- DNA (nucleus)

Cells

The cells you will be staining are **epithelial** cells. They are '**adherent**', meaning they stick to the glass slide. The cells will look like those in **Fig. 1**.

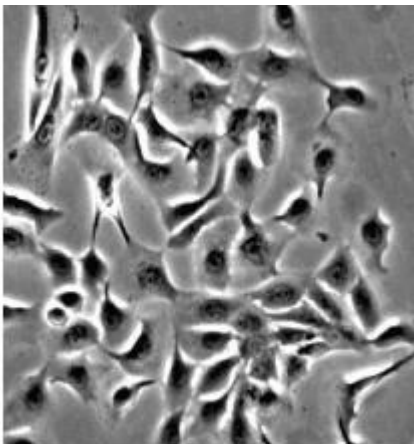


Fig. 1: Breast Cancer Epithelial Cells

Formaldehyde is the most commonly used cellular fixative. A fixative chemically bonds adjacent macromolecules, such as proteins, together. This preserves and strengthens cell structure and strengthens the cells so they can withstand further processing. Fixing also kills the cells, making them safe to work with outside of a tissue culture hood (**Fig 2**).

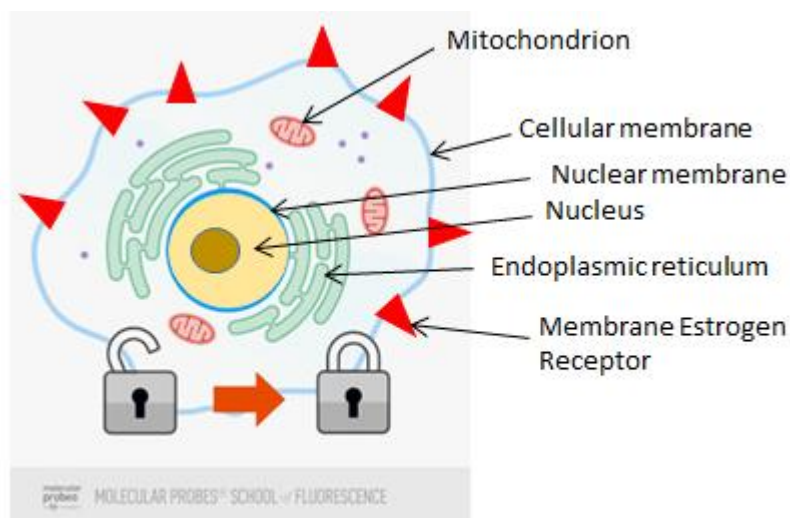


Fig 2. Fixation locks cellular structures in place (Ref b).

Protocol:

Part 1: Obtaining Cells

1. Obtain a chamber slide (**Fig 3**) in which one of the chambers contains fixed patient cells. The chamber containing cells will have Wash Buffer in it (1X phosphate buffered saline, or 'PBS'). The camp instructors will demonstrate how to properly use the inverted microscopes that are on the lab benches.
2. Examine the patient cells using the microscope.
3. The slide will be labeled with the patient ID letter. Add your initials to the slide as well.
4. Remove the 1 mL Wash Buffer from the slide chamber using a P1000 micropipette. Discard the buffer into a small waste beaker.



Fig 3: Chamber Slide

Part 2: Staining for membrane-bound Estrogen Receptor (mER)

These steps will allow antibodies that are specific for **mER** to bind to any mER that is on the cell membrane (**Fig 4**).

5. Add 400uL of the solution containing the anti-mER mAb conjugated to Texas Red (labeled **mER**).
6. Cover the slide with foil and incubate at room temperature for 10 minutes
7. Remove the 400uL of anti-mER mAb solution with a P1000 and discard it in the waste beaker.
8. Wash the cells by carefully adding 400uL of Wash Buffer. Wait 2 minutes.
9. Remove the 400uL of Wash Buffer with a P1000 and discard it in the waste beaker.
10. Repeat steps 8 and 9 two more times, for a total of 3 washes.

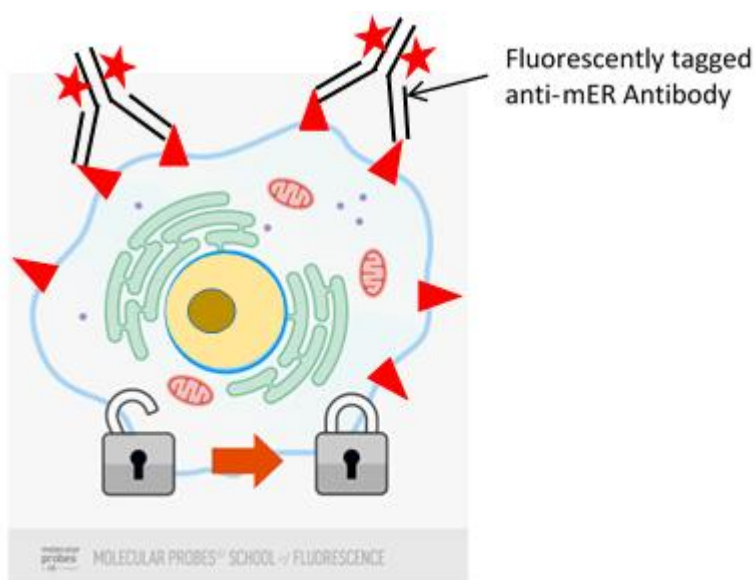


Fig 4. Fluorescently labeled antibodies can be used to detect expression of proteins on the cell membrane.

Part 3: Cell Permeabilization

The **permeabilization** step removes more cellular membrane lipids to allow large molecules like antibodies to get inside the cell. Detergents are commonly used for permeabilization. These detergents will also permeabilize the nuclear membrane (**Fig 5**).

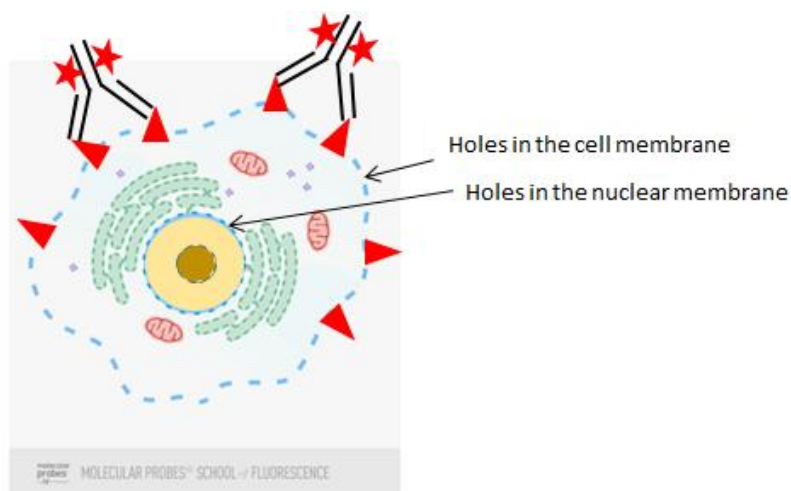


Fig 5. Permeabilization creates holes in the membranes, allowing other molecules to enter the cell.

11. Add 400uL of Permeabilizing Solution (labeled **Perm**).
12. Cover the slide with foil and incubate at room temperature for 15 minutes. .
13. Remove the 400uL of Permeabilizing Solution with a P1000 and discard it in the waste beaker.
14. Wash the cells by carefully adding 400uL of Wash Buffer. Wait 2 minutes.
15. Remove the 400uL of Wash Buffer with a P1000 and discard it in the waste beaker.
16. Repeat steps 14 and 15 two more times, for a total of 3 washes.

Part 4: Blocking

Blocking with a solution containing an excess of protein can help reduce non-specific background staining. If there are areas in the cell that are just generally sticky, the proteins in the blocking solution will bind there. Now when you add your dye or antibody that is specific for a particular cellular organelle or structure, the dye or antibody will be less likely to non-specifically stick to other areas of the cell.

17. Add 400uL of Blocking Solution (labeled **Block**).
18. Cover the slide with foil and incubate at room temperature for 30 minutes.
19. Remove the 400uL of Blocking Solution with a P1000 and discard it in the waste beaker.
20. Wash the cells by carefully adding 400uL of Wash Buffer.
21. Remove the 400uL of Wash Buffer with a P1000 and discard it in the waste beaker.
22. Repeat steps 19 and 20 two more times, for a total of 3 washes.

Part 5: Staining Actin (cytoskeleton)

ActinGreen is a selective actin probe that is conjugated to a green-fluorescent dye. This probe can enter the permeabilized cells through the pores in the cell membrane formed during permeabilization (**Fig 6**).

23. Add 400uL of the Actin Green solution (labeled **Actin**).
24. Cover the slide with foil and incubate at room temperature for 30 minutes.
25. Remove the 400uL of Actin Green solution with a P1000 and discard it in the waste beaker.
26. Wash the cells by carefully adding 400uL of Wash Buffer.
27. Remove the 400uL of Wash Buffer with a P1000 and discard it in the waste beaker.
28. Repeat steps 25 and 26 two more times, for a total of 3 washes.

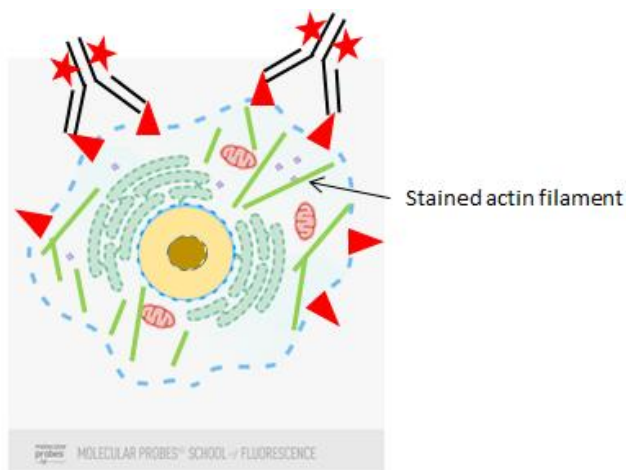


Fig 6. ActinGreen can enter through the holes in the cell membrane to detect the actin filaments of the cytoskeleton.

Part 6: Staining DNA in the Nucleus

NucBlue contains **Hoechst dye**, which binds to nucleic acids and stains cell nuclei. It can enter the cell and then the nucleus through the pores in these membranes formed during cell permeabilization (**Fig 7**).

29. Add 400uL of the NucBlue Solution (labeled **Nuc**).
30. Cover the slide with foil during and incubate at room temperature for 20 minutes.
31. Do not wash after this step.
32. Image the stained cells using the EVOS FL fluorescent microscope. The camp instructors will operate the microscope.
33. For storage, add 1mL of Wash Buffer to the chamber containing your stained cell sample. Carefully wrap the slide in plastic wrap and cover it with foil. Store the slide in the refrigerator.

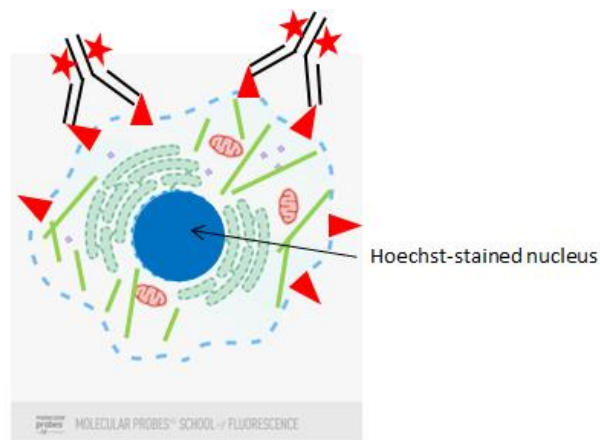


Fig 7. Hoechst stain can enter through holes in the cell and nuclear membranes and bind to the nucleic acids (DNA) in the nucleus.

References

- a) <https://breast-cancer-research.biomedcentral.com/articles/10.1186/bcr1275>
- b) <https://www.thermofisher.com/us/en/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/imaging-basics/sample-considerations/preparing-fixed-cells-imaging.html>