

Name:

Date:

Period:

Modeling Cell Staining: Using fluorescent stain to detect over-expressed mER
Shoreline Biotech Experience Cancer Biology Kit

Background:

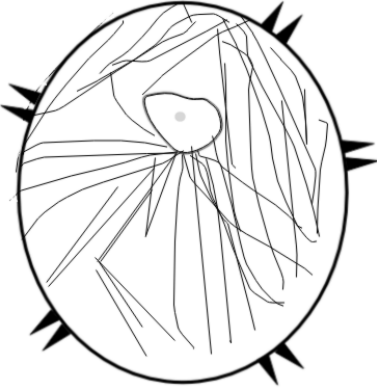

Possible cancer patient cells may be tested using a cell staining protocol. The test shows the level of expression of membrane-bound Estrogen Receptor (mER) using an mER-specific antibody that is bound to a red fluorescent molecule. Normal cells typically express low levels of mER. High levels of mER on a cell's surface can be a driver in the development of breast cancer. Keep in mind that not all breast cancers are caused by high expression of mER. Some breast cancer cells express normal (low) amounts of mER. Use the following protocol to model results of cells from breast cancer patients that test positive or negative for over-expression of mER on their cell membranes.

For mER staining:

Negative result = VERY low mER on cell surface

Positive result = lots of mER on cell surface (over-expression) = Significant red detection using fluorescence microscope

Compare results of Staining

Breast Cancer Cell expressing normal levels of mER [Low levels mER]	Breast Cancer Cell over-expressing mER [High Levels mER]
	
Staining protocol:	How to model:
A. Stain the cell membrane with an antibody (Ab) that is specific for mER. This will have a red fluorescent tag. NOTE: Excess Ab is washed away during this step	A. Draw 'Y' shaped Ab molecules binding to all mER proteins found on each cell. Be sure to draw "arms" of the 'Y' to the antibody. Draw a red star on the other end of the 'Y' Ab symbol to represent the red fluorescent tag.
B. Permeabilize the cell (using a mild detergent solution) to allow the stain that binds to actin and the stain that binds to DNA to enter the cell. Cells are again washed after this step.	B. Use white out or similar to represent holes the form in both the cell membrane and the nuclear membrane.
C. Add the actin stain, which is labeled with a green fluorescent protein. Excess stain will be washed away.	C. Stain the actin filaments green throughout the cytoplasm of the cell.
D. Add the nucleic acid stain, which fluoresces blue.	D. Color the nucleus with blue pencil.
E. Staining is complete.	

Analysis

- 1) Compare the two cells.
- 2) When observing actual slides, what do you think a cell with very low levels of mER would look like?
- 3) What does it likely mean if cells are brightly stained red?
- 4) Which cell parts are clearly visible on both types of cells?
- 5) How do you distinguish a breast cancer cell that expresses 'normal' levels of mER from a breast cancer cell that over-expresses mER using a fluorescent microscope?
- 6) How could you modify this fluorescent staining protocol to look for the over-expression of other receptors that can lead to the development of other types of cancer?