Antibody + TO-PRO-3 Staining Protocol (from Molecular Probes website)

Sample Preparation

(All steps are carried out at room temperature unless otherwise noted)

Sample Preparation

1. Remove media from live cells, wash in PBS, and fix cells for 15 minutes in 3.7% formaldehyde solution at 37°C.

2. Wash cells 3 x 5 minutes in PBS (1X).

3. Permeabilize cells by treating for 10 minutes with PBT (0.1% Triton X-100 in PBS (1X)).

4. Wash cells 3 x 5 minutes in PBS.

Staining

1. Block cells for 1 hour in antibody blocking buffer (BlockAid Blocking Solution).

2. Incubate cells with 5 µg/ml of anti-OxPhos Complex V inhibitor protein primary antibody in BlockAid Solution for 1 hour.

3. Wash cells 3 x 10 minutes in PBT.

4. Incubate cells with 5 µg/ml Alexa Fluor 555 goat anti-mouse IgG in 1% BSA/PBT for 1 hour.

5. Wash 3 x 10 minutes in PBS.

6. Incubate cells for 20 minutes with Alexa Fluor 488 phalloidin, diluted 1:200 from stock solution.

7. Wash 3 x 5 minutes in PBS.

8. Label cells with 1 µM TO-PRO®-3 dye in PBS for 15 minutes.

9. Wash 3 x 10 minutes in PBS.

10. Mount the specimen in ProLong Gold antifade reagent.
Mounting Reagent Preparation and Sample Processing

1. Remove the ProLong Gold antifade reagent from the freezer and allow the vial to equilibrate to room temperature. Using an external heat source to warm the vial is not recommended, as this may decrease the long-term stability of the product.

2. Remove any excess liquid from the specimen and apply 1 or 2 drops (depending on the surface area of your sample) of the antifade reagent to the specimen. Cover slide-mounted specimens with a coverslip; for specimens mounted on coverslips, place a drop of antifade reagent onto a clean slide and carefully lower the coverslip onto the antifade reagent to avoid trapping any air bubbles.

3. Allow the mounted sample to cure on a flat surface in the dark. Curing time may vary from a couple of hours to overnight, depending on the thickness of the sample and the relative humidity of the surrounding air. For long-term storage, seal the coverslip to the slide after curing to prevent excessive shrinkage of the mounting medium, which can result in sample distortion. After sealing, store the slide upright in a covered slide box at <=-20°C. Desiccant may be added to the box to ensure that the slide remains dry.

To view the samples immediately, secure the coverslip at the corners using nail polish or hot wax to prevent the coverslip from moving. Leave the edges clear to allow the preparation to cure.

Note: The antifade properties of ProLong Gold antifade reagent improve slightly the longer it remains in contact with the specimen. To further reduce photobleaching, minimize the exposure of fluorescently labeled specimens to light by using neutral density filters, and expose samples only when observing or recording a signal. Optimize image capture by using a minimum of optics, high-numerical aperture objectives, relatively low magnification, high-quality optical filters, and high-speed film or high-efficiency detectors.

To further reduce photobleaching, minimize the exposure of fluorescently labeled specimens to light by using neutral density filters and expose samples only when observing or recording a signal. Optimize image capture by using a minimum of optics, high-numerical aperture objectives, relatively low magnification, high-quality optical filters, and high-speed film or high-efficiency detectors.