1. Fix cells in 3% Paraformaldehyde in PBS for 15 minutes at room temperature, gently rocking.
2. Rinse cells 3 times for 5 minutes in PBS.
3. Block and permeabilize cells in 2% non-fat dry milk (NFDM) dissolved in PBS with 0.1% TX-100 overnight at 4C (covered to prevent evaporation).
4. Rinse cells 3 times for 5 minutes in PBS.
5. Dilute NB100-123 1:500 in dilution buffer [2% BSA in PBS with 0.01% TX-100].
6. Place cover slip upside down on a 50 ul drop of diluted antibody on parafilm, in humidity box.
7. Incubate for 1 hour at 37C.
8. Flip slips right side up in wells and rinse 3 times for 5 minutes each, in PBS.
9. In an amber microfuge tube, dilute secondary antibody (Cy3 anti-ms IgG) 1:500 in dilution buffer [2% BSA in PBS with 0.01% TX-100].
10. Place 800 ul of diluted secondary antibody in each well and make sure the fluid film covers over the cells on the slip. Alternatively, secondary antibody can be applied in the same manner as the primary (slip upside-down on drop of secondary that has been placed on a sheet of parafilm that is inside of a humidity box).
11. Incubate for 1 hour at 37C, in the dark.
12. Rinse cells at room temperature 4 times for 15 minutes each, in PBS, gently rocking.
14. Refrigerate flat and covered.