

# PRODUCT-SPECIFIC PROTOCOLS

## IF (25654-1-AP)

| Sample type | Fixation method                    | Blocking buffer                                             | Antibody dilution buffer    | Primary antibody dilution | Incubation time                      |
|-------------|------------------------------------|-------------------------------------------------------------|-----------------------------|---------------------------|--------------------------------------|
| A375 cells  | -20°C Ethanol, 10 min at room temp | 5% goat serum in 1×PBS                                      | 1% BSA in 1×PBS             | 1:50                      | 1.5 h at room temp                   |
|             |                                    | Secondary antibody                                          | Secondary antibody dilution | Incubation time           | Mounting media                       |
|             |                                    | Coralite488-Conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) | -                           | 1.5 h at room temp        | Hydromount? mounting media with DAPI |

# PROTOCOL (more details available upon request):

1. Seed cells on sterile glass coverslips and gently wash the coverslip with 1X PBS.
2. Fix the samples with -20°C Ethanol, 10 min at room temp.
3. Rinse coverslip with 1XPBS 3 times for 5 min each.
4. Incubate the samples with 0.2% Triton X-100 made in 1XPBS for 5 min at room temperature.
5. Rinse the samples 3 times with 1XPBS for 3 min each.
6. Incubate the samples with Blocking buffer at room temperature for 1 h.
7. Aspirate Blocking buffer and incubate with primary antibody at room temperature for 2 hours or at 4°C for overnight.
8. Wash samples with 1XPBS 3 times for 5 min each.
9. Incubate with fluorochrome-labeled secondary antibody diluted in Antibody dilution buffer.
10. Wash coverslip with 1XPBS 3 times for 3 min each.
11. Mount and seal the sample, then visualize the target antigen using a fluorescent microscope.

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