

# PRODUCT-SPECIFIC PROTOCOLS

## IF (17590-1-AP)

| Sample type                                  | Fixation method                      | Blocking buffer                                               | Antibody dilution buffer    | Primary antibody dilution | Incubation time                      |
|----------------------------------------------|--------------------------------------|---------------------------------------------------------------|-----------------------------|---------------------------|--------------------------------------|
| PMA, LPS and Brefeldin A treated THP-1 cells | 4% PFA in 1×PBS, 10 min at room temp | 5% goat serum in 1×PBS                                        | 1% BSA in 1×PBS             | 1:200                     | 1.5 h at room temp                   |
|                                              |                                      | Secondary antibody                                            | Secondary antibody dilution | Incubation time           | Mounting media                       |
|                                              |                                      | Coralite®488-Conjugated Goat Anti-Rabbit IgG(H+L) (SA00013-2) | -                           | 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 4% PFA in 1×PBS, 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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