

# PRODUCT-SPECIFIC PROTOCOLS

## IF (CL488-84117)

| Sample type | Fixation method                      | Blocking buffer    | Antibody dilution buffer             | Primary antibody dilution | Incubation time    |
|-------------|--------------------------------------|--------------------|--------------------------------------|---------------------------|--------------------|
| U2OS cells  | 4% PFA in 1×PBS, 10 min at room temp | 3% BSA 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     |
|             |                                      | -                  | 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. Mount and seal the sample, then visualize the target antigen using a fluorescent microscope.

---

Visit [ptglab.com](https://ptglab.com) for full product range