

Protocol for IF
Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Revised in March, 2014)

1. **Baking:** Bake IHC slides for 0.5~2 hours at 60°C.
2. **Deparaffinization and rehydration:** After the baking, put slides into the following reagents in order:
 - 1) Xylene I: 30 minutes
 - 2) Xylene II: 30 minutes
 - 3) 100% ethanol: 10 minutes
 - 4) 95% ethanol: 10 minutes
 - 5) 80% ethanol: 10 minutes
 - 6) 70% ethanol: 10 minutes
 - 7) Rinse sections with running water for 10 minutes
3. **Antigen retrieval: - Microwave heat antigen retrieval (optional):** Transfer the slides into a boiling EDTA-TRIS solution, and ensure the slides are soaked completely. Low fire for one minute, stop heating and keep in microwave for 10min, then another low fire for 3-4 min, and allow slides to cool in the EDTA-TRIS solution at room temperature..
4. **Rinse 3X in PBS:** Remove the residual, soak in PBS, and rinse in shaker at 40 RAM, 5min each time.
5. **Blocking endogenous peroxidase:** Incubate the slides with 3%-5% H₂O₂ Solution for 10min at room temperature.
6. **Rinse 3X in PBS:** Remove the residual, soak in PBS, and rinse in shaker at 40 RPM, 5min each time.
7. **Blocking Nonspecific epitope:** Remove the residual, incubate the slide with 5% BSA solution for 20min at room temperature.
8. **Incubate with FITC-conjugated antibody:** Remove the residual, apply diluted FITC-conjugated antibody at a appropriate dilution for 100-120ul per slide, then incubate overnight at 4°C in the dark.
9. **Rinse 3X in PBST:** Remove the residual, soak in PBST, and rinse in shaker at 40 RAM, 5min each time.
10. **Nucleus staining:** apply DAPI staining solution on slides (~200 ul), incubate

15 min in dark.

11. Add 50% PBS and 50% glycerol to the slide and then observe specimens under a fluorescence microscope immediately.

Note:

1. Dewaxing is very important, and it has direct influence for the result. If dewaxing is not complete, it will cause a strong background of fluorescence, and the fluorescence can be seen without the addition of antibody.
2. As fluorescence can photobleach when exposed to light, so incubating with secondary antibody and the rinse process should be done in the dark.