

## ICC-IF PROTOCOL

Standard protocol for immunocytochemistry with fluorescent detection optimized for Triple A Polyclonals™ and PrecisA Monoclonals™ from Atlas Antibodies. In addition to Triple A Polyclonals and PrecisA Monoclonals a set of reference markers are also used: an anti-alpha tubulin antibody and the nuclear dye DAPI.

### SAMPLE PREPARATION

Perform all washes at room temperature.

1. Coat a multiwell, glass-bottomed plate (Greiner bio-one, product no: 655891) with fibronectin (concentration 12.5 µg/ml) for 1 h at room temperature.
2. Seed the cells (10,000-25,000 cells per well) and incubate at 37°C in humidified air with 5.0% CO<sub>2</sub>, for 20-24 h.

### IMMUNOSTAINING

3. Remove the growth medium and wash the cells in Dulbecco's Phosphate Buffered Saline (DPBS).
4. Fix for 15 min in 4% formaldehyde (methanol-free) in DPBS.
5. Permeabilize for 10 min with 0.1% Triton X-100 in DPBS.
6. Wash with DPBS once and incubate for 1 h at room temperature in blocking buffer (4% FBS in DPBS) to block non-specific sites.
7. Incubate the cells overnight at 4°C with primary antibodies in blocking buffer.
8. The following day, remove the primary antibodies and rinse the cells briefly in DPBS. Then wash with DPBS 3x10 min.
9. Incubate for 1.5 h at room temperature with secondary antibodies in blocking buffer. **Note:** *The secondary antibodies are fluorescently labeled and thus light sensitive. Keep the sample protected from light.*
10. Counterstain for 5 min with the nuclear stain DAPI (Invitrogen) at 1µg/ml in DPBS.
11. Wash with DPBS 3x10 min and mount in DPBS containing 0,02 % (w/v) NaN<sub>3</sub>.

### REAGENTS

#### PRIMARY ANTIBODIES:

- Triple A Polyclonals at a working concentration of 0.25-2 µg/ml.
- PrecisA Monoclonals at a working concentration of 2-10 µg/ml.

#### Reference markers

- Mouse anti-alpha Tubulin monoclonal antibody Ab7291 (Abcam) diluted 500x for Triple A Polyclonals.
- Rabbit anti-alpha Tubulin monoclonal antibody ab52866 (Abcam) diluted 1000x for PrecisA Monoclonals.

#### SECONDARY ANTIBODIES:

##### For Triple A Polyclonals

- Alexa® Fluor 488 goat anti-rabbit IgG (ThermoFisher Scientific, product no: A11034) diluted 400x.
- Alexa® Fluor 594 goat anti-mouse IgG (ThermoFisher Scientific, product no: A11032) diluted 400x.

##### For PrecisA Monoclonals

- Alexa® Fluor Plus 488 goat anti-mouse IgG (ThermoFisher Scientific, product no: A32723) diluted 800x.
- Alexa® Fluor 594 goat anti-rabbit IgG (ThermoFisher Scientific, product no: A11037) diluted 400x.

**NOTE:** *The dilution of the antibodies is to be considered as a guideline only. Optimal dilution must be determined by the user.*

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