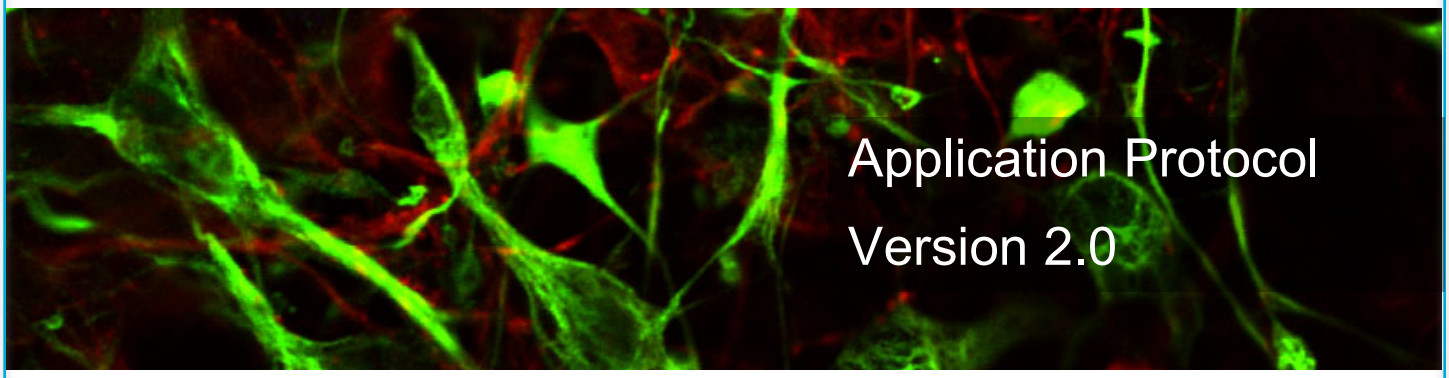
# Axol Guide to Performing Immunocytochemistry (ICC)



Application Protocol

Version 2.0

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# General ICC Protocol

## Sample Preparation and Fixation

1. Add cell culture-grade coverslips to wells.
2. Make 1X solution of **Axol Sure Bond™** from the 50X stock using PBS, e.g. 240 µL in 12 mL PBS.
3. Add enough 1X **Axol Sure Bond™** to each well to immerse the coverslips and incubate overnight at 37°C.
4. Wash coverslips with sterile $H_20$ 3 x 5 mins.
5. Grow cells on coated coverslips for desired length of time.
6. When ready to stain cells, rinse them briefly in PBS.
7. Fix the samples using 4% paraformaldehyde in PBS pH 7.4 for 15 minutes at room temperature.
8. Wash the samples twice with PBS.

## Cell Permeabilization & Blocking

1. To permeabilize the cells incubate the samples for 10 mins in PBS containing 0.3% Triton X-100.
2. Wash cells in PBS 3 x 5 mins.
3. To prevent non-specific antibody binding, incubate the samples for 1 hr with blocking buffer (5% serum from the species in which the secondary antibody was raised diluted in PBS e.g. 2.5 mL serum in 47.5 mL PBS).

## Staining

1. Dilute the primary antibody in blocking buffer using the dilution factor recommended by the antibody datasheet guidelines.

2. Incubate the cells in the primary antibody solution in a humidified chamber for 1 hr at room temperature or overnight at 4°C.

3. Dilute the secondary antibody in blocking buffer using the dilution factor recommended by the antibody guidelines.

4. Remove the primary antibody solution and then wash cells 3 x 5 mins with PBS.

5. Incubate the cells in the secondary antibody solution for 1 hr at room temperature in the dark.

6. Remove the secondary antibody and then again wash cells with PBS 3 x 5 mins in the dark.

## Mounting and Counter-Staining

1. Mount stained coverslip on slides using a drop of mounting medium containing DAPI (to counter stain the cell nucleus) according to manufacturer's guidelines e.g. ProLong Gold Antifade Reagent, Life Technologies.

2. Seal the edges of the coverslip with nail polish.

3. Store in the dark at 4°C.

**Top Tips:**

**1. For fixation and incubation steps use a rocker to ensure even distribution of fixative/antibody solutions.**

**2. For intracellular target proteins, cell permeabilization is essential**

**3. Triton X:100 disrupts membranes so do not use this with membrane-associated targets**

# Synaptic Marker ICC Protocol

**Follow the steps for sample preparation and fixation from the general ICC protocol before proceeding.**

## Cell Permeabilization & Blocking

1. Wash 3 times with 50 mM ammonium chloride.
2. Incubate for 5 mins with 50 mM ammonium chloride.
3. Incubate for 10 mins with 0.1% saponin in PBS.
4. Incubate for 30 mins in blocking buffer (PBS containing 3% BSA & 0.1% saponin).

## Staining

1. Dilute primary antibody in blocking buffer using manufacturer's recommended dilution.
2. Put a piece of parafilm on wet Whatman paper and apply 200 µL of primary antibody solution to the top of the parafilm.
3. Put coverslips upside down on primary antibody solution. Incubate for 1 hr at room temperature.
4. Transfer coverslips back to a tissue culture plate e.g. 12 well plate.
5. Wash twice with 0.1% saponin in PBS.
6. Incubate for 10 mins with blocking buffer.
7. While samples are in blocking, dilute secondary antibody in blocking buffer using manufacturer's recommended dilution.
8. Put a new piece of parafilm on wet Whatman paper and apply 200 µL of secondary antibody solution.

9. Put coverslips upside down on secondary antibody solution, as before so that cells are in contact with solution. Incubate for 1hr at room temperature.

10. Transfer coverslips to your tissue culture plate e.g. 12 well plate.

11. Wash twice with 0.1% saponin in PBS.

12. Wash twice with PBS.

## Mounting and Counter-Staining

1. Mount stained coverslip on slides using a drop of mounting medium containing DAPI (to counter stain the cell nucleus) according to manufacturer's guidelines e.g. ProLong Gold Antifade Reagent, Life Technologies.

2. Seal the edges of the coverslip with nail polish.

3. Store in the dark at 4°C.

## Recommended Markers

The following list includes the primary antibodies that can be used for characterizing neurons and astrocytes derived from Axol hNPCs:

Tuj1
Neuronal Marker (axon), Abcam ab14545

MAP2
Neuronal Marker (dendrite), Novus NB300-213

Doublecortin
Newborn Neuron, Abcam ab18723

Tbr1
Deep-layer Cortical Neurons, Abcam ab31940

Brn2
Upper-layer Cortical Neurons, Santa Cruz sc-6029

VGlut1
Glutamatergic Neurons, Synaptic Systems 135303

S100
Astrocytes, Dako Z0311

PSD-95
Postsynaptic Terminals, Abcam ab2723

Synaptophysin
Presynaptic Terminals, Abcam ab68851

# Technical Support

- ## Online Resources

  Please visit our website at www.axolbio.com for additional product information and *Technical Resources*, including instruction manuals, application protocols, video guides, wall charts and webinars.

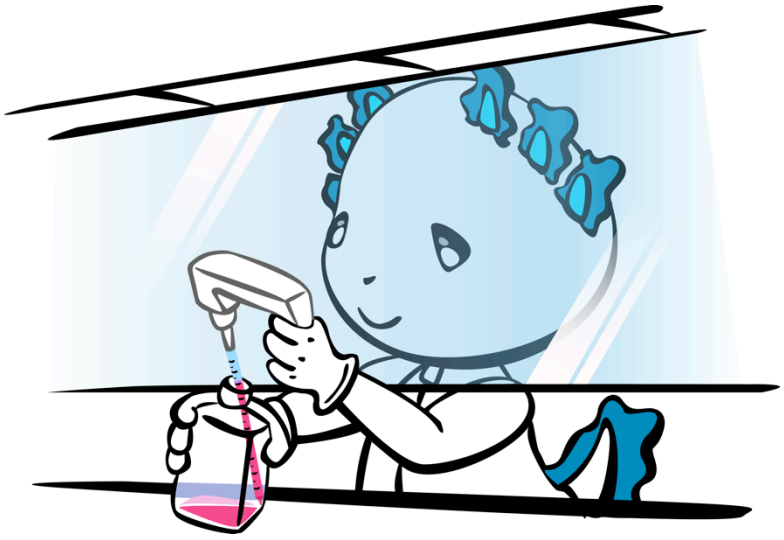
- ## Contact Us

  For more information or technical assistance, call +44 (0) 1223 497 119, or email support@axolbio.com. US Toll Free Tel: 1-800-678-2965 (1-800-678-AXOL), US Toll Free Fax: 1-800-861-2965 (1-800-861-AXOL).

- ## Certificate of Analysis

  The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available on our website.

  Go to www.axolbio.com/certificate-of-analysis-lookup and search for the Certificate of Analysis with product lot number, which is printed on the cryovial label.



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