Antibody Validation Protocol

Resources:

Relevant articles are available for download from http://www.chunglab.org/publications

Other protocols and resources are available at http://chunglabresources.com/

Antibody validation data can be found at https://www.molecularprobes.org/

Reagents:

Blocking Buffer

Combine the following:

- 150µL Triton X-100
- 2.5mL normal goat serum
- Fill with PBSN up to 50mL

Vortex to mix and store at 4°C.

Sodium Citrate Buffer*

Combine the following:

- 10mM Sodium Citrate
- 0.05% Tween 20
- Adjust to pH 6.0

*For antigen retrieval in human samples only

Choosing Antibodies

A typical validation experiment will contain, within one tissue, one primary antibody in need of validation and its corresponding secondary, and a previously validated antibody to the same target and its corresponding secondary.

Primary:

To validate a primary antibody, choose two primary antibodies to the same target. These antibodies must be produced in different hosts than each other.

Secondary:

The secondary antibody must correspond to the host of the primary antibody it is targeting. If using F(ab) fragments, the isotype must be matched as well. Since two primary antibodies are being used in the same tissue, their hosts must be different and their fluorophores must not overlap. Fluorophores at opposite end of the spectrum, e.g. 488 and 647, are recommended to minimize potential crosstalk.

Tissue Preparation:

Antigen Retrieval in Human Samples:

Incubate samples in sodium citrate buffer 30 min at 70C before proceeding with the protocol as usual.

Co-staining:

Primary Antibody Addition

1. Using the 24-well plate, fill each chamber with a small amount of PBST.
2. Transfer each tissue sample (150µm slices) into its own well using a 10mm tip brush.
3. Remove all PBST from each well.
4. Change pipette tip and refill each well with 300µL blocking buffer.
5. Add 1µg each of desired primary antibodies, being sure to centrifuge antibody tubes before opening.
6. Gently mix buffer solution 3-4 times with pipette.
7. Before fully covering the plate for incubation, check slices to ensure they are free-floating by putting on the plate lid and gently shaking. If a sample doesn't move, use a pipette tip to gently dislodge.
8. Cover the 24-well plate with an adhesive plate seal and the plate lid.
9. Gently shake overnight on orbital shaker at room temperature.

Primary Antibody Washing

1. Remove 24-well plate from shaker.
2. Remove the solution from each well, tilting the plate to make sure you remove every last drop of solution
   *Always change the pipette tip after each removal to prevent contamination.
3. Add a minimum of 1mL PBST to each well for washing.
4. Before fully covering the plate for incubation, check slices to ensure they are free-floating.
5. Cover the 24-well plate adhesive plate seal and plate lid.
6. Shake for 1 hour minimum on orbital shaker at room temperature.
7. Repeat two more times for a total of three washes.

Secondary Antibody Addition

1. Remove all solution from each well.
2. Add 300ul fresh blocking buffer to each well.
3. Add 1µg desired fluorescent-dye conjugated secondary antibodies, where the secondary antibody corresponds to the hosts of the primary antibodies. Be sure to centrifuge antibody tubes before opening.
4. Addition of a nuclear counterstain (e.g. DAPI) is recommended.
5. Gently mix buffer solution 3-4 times with pipette.
6. Before fully covering the plate for incubation, check slices to ensure they are free-floating by covering the plate and gently shaking.
7. If sample doesn't move, use a pipette tip to gently dislodge

8. Cover plate with aluminum foil
9. Gently shake overnight on orbital shaker at room temperature.

Secondary Antibody Washing

1. Remove 24-well plate from shaker.
2. Remove the solution from each well, tilting the plate to make sure you remove every last drop of solution
   *Always change the pipette tip after each removal to prevent contamination.
3. Add a minimum of 1mL PBST to each well for washing.
4. Check slices to ensure they are free-floating.
5. Cover the plate with an adhesive plate seal and plate lid.
6. Cover plate with aluminum foil
7. Shake for 1 hour minimum on orbital shaker at room temperature.