Immunohistochemistry Protocols

Immunofluorescence of slide-mounted sections
Tissue is fixed and sectioned frozen. Sections are thaw-mounted onto gelatin coated slides. After tissue mounting, slides are warmed on a slide warmer. Slides are stored frozen at -20ºC.

Reagents

- Primary Antibody: specific for protein of interest
- Fluorescently labeled secondary antibody specific for the species used to raise the primary antibody.

  Recommended: secondary antibodies from Jackson ImmunoResearch
  For antisera raised in guinea pigs: donkey anti-guinea pig labeled with FITC (Jackson ImmunoResearch Laboratories, cat # 706-095-148) or Cy3 (Jackson ImmunoResearch Laboratories, cat # 706-165-148).

  For antisera raised in rabbits: donkey anti-rabbit labeled with FITC (Jackson ImmunoResearch Laboratories, cat # 711-095-152) or Cy3 (Jackson ImmunoResearch Laboratories, 711-165-152).

Solutions

- Phosphate buffered saline pH 7.45 (PBS)
- Blocking Buffer: phosphate buffered saline containing 0.3% Triton X-100, 1.0% bovine serum albumin, 1-3.0% normal donkey serum and 0.01% sodium azide, pH 7.45

Immunofluorescence protocol
Care should be taken so that the incubation solutions do not evaporate. It is recommended that incubations be performed in a humidified chamber to prevent evaporation. Washing steps should be done in large volumes of phosphate buffered saline (PBS).

- Warm slides to room temperature.
- Re-hydrate with PBS for 10-15 minutes.
- Remove PBS and incubate in blocking buffer for one hour at room temperature.
- Dilute primary antibody in blocking buffer. Store diluted antiserum at 4ºC until use.
- Remove blocking buffer and add diluted primary antibody.
- Incubate with primary antibody at 4º C for 18-48 hours.
- Remove primary antibody and wash three times for 10 minutes with PBS.
- Dilute secondary antibody in blocking buffer to appropriate dilution (follow manufacture's recommendation). Store at 4ºC until use.
- Incubate slides in diluted secondary antibody solution for one hour at room temperature.
- Remove secondary antibody and wash three times for 10 minutes with PBS.
- Coverslip slides with appropriate mounting media.

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Immunofluorescence of free-floating sections
Tissue is fixed and sectioned frozen. Sections are stored in PBS or blocking buffer with 0.01% sodium azide. Store at 4ºC until use.

Reagents

- Primary Antibody: specific for protein of interest
- Fluorescently labeled secondary antibody specific for the species used to raise the primary antibody.

  Recommended: secondary antibodies from Jackson ImmunoResearch
  For antisera raised in guinea pigs: donkey anti-guinea pig labeled with FITC (Jackson ImmunoResearch Laboratories, cat # 706-095-148) or Cy3 (Jackson ImmunoResearch Laboratories, cat # 706-165-148).

  For antisera raised in rabbits: donkey anti-rabbit labeled with FITC (Jackson ImmunoResearch Laboratories, cat # 711-095-152) or Cy3 (Jackson ImmunoResearch Laboratories, 711-165-152).

Solutions

- Phosphate buffered saline pH 7.45 (PBS)
- Blocking Buffer: phosphate buffered saline containing 0.3% Triton X-100, 1.0% bovine serum albumin, 1-3.0% normal donkey serum and 0.01% sodium azide, pH 7.45

Immunofluorescence protocol
Washing steps should be done in large volumes of phosphate buffered saline (PBS).

- Incubate in blocking buffer for one hour at room temperature.
- Dilute primary antibody in blocking buffer. Store diluted antiserum at 4ºC until use.
- Remove blocking buffer and add diluted primary antibody.
- Incubate with primary antibody at 4º C for 48-72 hours.
- Remove primary antibody and wash three times for 1 hour with PBS at room temperature.
- Dilute secondary antibody in blocking buffer to appropriate dilution (follow manufacture's recommendation). Store at 4ºC until use.
- Incubate in diluted secondary antibody solution at 4º C for 24-36 hours.
- Remove secondary antibody and wash three times for 1 hour with PBS at room temperature.
- Mount onto gel-coated slides and dry at room temperature.
- Coverslip slides with appropriate mounting media.

Preabsortption of Antibody with Blocking Peptide

Antibody should be pre-incubated with peptide prior to incubation with tissue/sample.

- Pre-incubate primary antibody with blocking peptide for 1 hour at room temperature in blocking buffer. A 10^{-6} or 10^{-5} molar (M) peptide concentration is suggested to block antigen/antibody binding.
- Follow immunohistochemistry for normal staining.

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