

## **Suggested Protocol for Immunohistochemistry**

1. Deparaffinization and hydration
  - a. Incubate the slides in a dry oven at 60°C for 1 hour. Allow the slides to cool to room temperature.
  - b. Dewax by immersing the slides in Xylene, 2 x 10 min.
  - c. Hydrate the arrays in 100% ethanol for 5 minutes, in 95% ethanol for 5 minutes and in 75% ethanol for 5 minutes.
  - d. Rinse the slides with DI water for 2 minutes.
2. Antigen retrieval if necessary.
3. Quenching endogenous peroxidase (optional)
  - a. Immerse the slides in 3% hydrogen peroxide solution for 5 min.
  - b. Wash the slides in PBS for 5 min, three times.
4. Primary antibody
  - a. Incubate sections with blocking serum for 30 min.
  - b. Discard the excess serum from sections.
  - c. Rinse the slides with PBS, 5 x 1 min.
  - d. Incubate sections with diluted primary antibody for 30 min at 37°C or overnight at 4°C.
  - e. Rinse the slides with PBS, 3 x 5 min.
5. Secondary antibody
  - a. Incubate sections with biotin-conjugated secondary antibody for 30 min at 37°C.
  - b. Wash the slides with PBS, 3 x 5 min.
6. Avidin-Biotin-peroxidase complex
  - a. Incubate section with Avidin-Biotin Complex for 30 min at 37°C.
  - b. Wash the slides with PBS, 3 x 5 min.
7. Chromomeric reaction
  - a. Incubate sections in fresh chromogen (for example, DAB) for not more than 10 min
  - b. Rinse the slides with DI water.
8. Counter stain
  - a. Incubate section in counter stain solution (for example, hematoxylin) for 10 seconds.
9. Dehydration, clearing and mounting
  - a. Dehydrate the slides in 75%, 80%, 95% and 100% ethanol for 5 min each.
  - b. Immerse the slides in xylene for 5 minutes, 3 times.
  - c. Mount coverslips.
  - d. The arrays are now ready for viewing under microscope.