Frozen Immunohistochemistry Operation Protocol

1. Prepare 5-8 μm frozen tissue sections with adhesive slides. Immerse the slides immediately in Celnovte fixative for 30-60 seconds.

2. Rinse the slides in water for 20-30 seconds to remove fixative and embedding medium. Circle the tissue with a PAP pen, then rinse the slides with wash buffer.

3. Shake off excess wash buffer, add blocking reagent and incubate for 30 seconds. Rinse off the blocking reagent with wash buffer approximately three times within 20-30 seconds.

Note: Gently flush the tissue with a wash bottle during rinsing to remove all bubbles generated during blocking incubation.

4. Shake off excess wash buffer, add the corresponding primary antibody and incubate for 4-5 minutes. Rinse with wash buffer for 1 minute with no less than three times of flushing.

5. Shake off excess wash buffer, add polymer secondary antibody and incubate for 3-4 minutes. Rinse with wash buffer for 1.5 minutes with no less than three times of flushing.

6. Shake off excess wash buffer, add 100 μL freshly prepared DAB mixture (DAB substrate : DAB buffer = 1:20) and incubate for 1.5 minutes. Rinse with purified water, followed by a brief rinse with wash buffer.

7. Shake off excess wash buffer, add hematoxylin and incubate for 15-20 seconds, then rinse with purified water.

8. Perform dehydration, clearing and slide mounting.

Note: The wash buffer is 10× concentrated. Dilute it with purified water at a ratio of 1:9 before use.