Detection of Beta Catenin in Formalin-Fixed,
Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
Normal Rabbit IgG – Affinity Purified
DAB Chromogen
Hematoxylin

Staining Kit: ImmPRESS Anti-Rabbit Ig (peroxidase) Polymer Detection Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # MP-7401

Note: This kit contains a pre-dilute blocking serum and polymer reagent.

Primary Antibody: Rabbit Polyclonal Anti-Beta Catenin Antibody (H-102)
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog # sc-7199
**Staining Procedure**

Positive Control Tissue: Liver or gastrointestinal tract (GI tract may require a weaker primary dilution.)

Stain Localization: Cell membrane

1. Deparaffinize and hydrate slides through the following solutions:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Repetitions</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylene</td>
<td>2 times</td>
<td>5 minutes</td>
</tr>
<tr>
<td>100% Ethanol</td>
<td>2 times</td>
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2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

4. **Heat-Induced Epitope Retrieval Using The Decloaker**
   
   Add 500 ml of distilled water to the pan inside the decloaker. All three of the decloaker’s containers must be filled. Any containers without samples should have 250 ml of distilled water. The samples need to be in a container with a full rack of slides and about 200 ml of **1X citrate buffer**. (Insert blank slides into any empty slots in the rack to ensure even heating of slides.)
   
   Decloak the slides for 15 minutes at 110°C. **Maximum Pressure ________**
   
   Remove pan top and cool for 10 minutes. **Temperature Before Cooling Slides ________**
   
   Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

6. Block with the 2.5% normal horse serum from the ImmPRESS kit for 20 minutes at room temperature.
   
   **Lot #_________________ Exp. Date_________________**

   **DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.**
   **ONLY WIPE EXCESS BLOCK.**

7. Apply primary antibody at a 1:350 dilution. Incubate for 1 hour at room temperature.
   
   **Lot #_________________ Exp. Date_________________**

   For negative control slides, dilute normal rabbit IgG so that it’s IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:350 dilution. If the concentrations can’t be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.
   
   **Lot #_________________ Exp. Date_________________**

8. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

9. Apply the anti-rabbit polymer from the ImmPRESS kit, and incubate for 30 minutes at room temperature.
   
   **Lot #_________________ Exp. Date_________________**
10. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

11. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.
   (Add 1 drop of DAB per ml of substrate)
   Lot #________________ Exp. Date_________________ New Kit: yes / no

12. Rinse the slides in tap water 3 minutes.

13. Counterstain with hematoxylin for 20 seconds.

14. Rinse the slides in tap water until water is clear.

15. Gently agitate slides in 1X wash buffer until the tissues turn blue.

16. Dehydrate through the following solutions:

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17. Coverslip

*Updated 01/31/13*