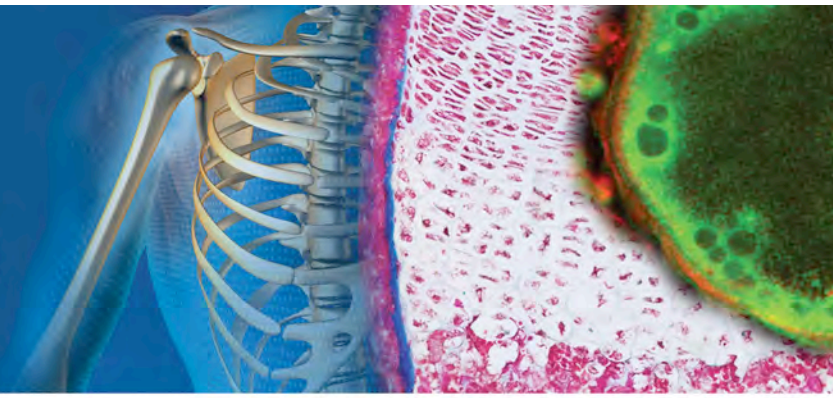

β-Catenin

Cell Signaling Cat # 9562
Immunohistochemistry Protocol For Formalin Fixed Paraffin Embedded Tissue

Cut sections at 3 microns and bake overnight at 60°C

Day 1

1. Bake slides at **60°C for minimum of 30 minutes** prior to staining.
2. Deparaffinize tissues in xylene (3 changes for 5 minutes each), and rehydrate through 2 changes in 100% Ethanol, 2 changes in 95% Ethanol and 1 change in 70% Ethanol for 5 minutes each).
3. Wash twice in deionized water for 5 minutes each.
4. Perform antigen retrieval using a **0.01 M (10mM) NaCitrate Buffer pH 6.0** – This is done in the water bath at a constant temp. **Set water bath temp to 65°C** – leave slides in a stain bucket in the water bath for **2 Hours.** After this step leave the bucket outside to cool for 20 minutes.
5. Rinse the slides in 3 changes of deionized water.
6. Outline each section with a **PAP pen.**
7. Quench endogenous peroxidase activity with **DAKO dual endogenous enzyme blocking reagent (***Dako S2003***) for 30 min.**
8. Rinse the slides in 2 changes of deionized water and once in 1X PBST.
9. Block non-specific binding sites **the diluted Vectastain Normal Goat Serum (***Vectastain Elite Rabbit IgG Kit PK-6101)* **– incubate for 30 min.**
Do not rinse slides after the incubation period.
10. Block endogenous avidin in the tissue with **Vector Avidin reagent**. (*Vector SP-2001*) for 15 minutes.
11. Rinse twice with 1X PBST.
12. Block endogenous biotin in the tissue with **Vector Biotin reagent.** (*Vector SP-2001*) for 15 minutes.
13 . Rince twice with 1X PBST.
14. Prepare the primary antibody in **2% Normal Goat Serum,** this decreases non-specific staining. Negative control slides need to be incubated with 2% normal goat serum.
15. Incubate the slides overnight **at 4°C** with a **1:200 dilution of β-catenin primary antibody** (*Cell Signaling Cat# 9562)* **for 2-3 month old adult sections**.
Use a **1:400 dilution for embryo (E18.5) sections**

Day 2

1. Let slides warm up to room temperature for 15-30 minutes.
2. Wash the slides 5 times with 1X PBST for 5 minutes each.
3. Incubate with **Vectastain Biotinylated Goat anti-rabbit** secondary antibody **(***Vectastain Elite Rabbit IgG Kit PK-6101)* for **30 min.**
4. 4. Wash the slides 5 times with 1X PBST for 5 minutes each.
5. Reconstitute the **Vectastain ABC reagent** and incubate at **room temperature for 30 min.**
6. Incubate with **Vectastain ABC reagent** for **30 min.**
7. Wash the slides 4 times with 1X PBST, then twice in deionized water.
8. Detect color reaction with **Vector Immpact DAB** *(Vector SK-4105)* for a few minutes (check under microscope) until staining intensity is optimal.
9. Stop the reaction with deionized water.
10. Counterstain the sections with **Hematoxylin (***Zymed Cat # 93-3943)* for 5 minutes.
11. Rinse in tap water.
12. Place slides in 1X PBS for 1-3 minutes.
13. Rinse with deionized water.
14. Dehydrate quickly through 3 changes of 95% ethanol and 2 changes of 100% ethanol.
15. Clear in 3 changes of xylene and mount with Cytoseal.

Buffers: 10mM Na Citrate, 0.05% Tween-20 Buffer; pH 6.0 (Used for heat-induced epitope/antigen retrieval)

1. Tri-Sodium Citrate Dihydrate - 2.94g
2. Deionized water - 1000 ml
Mix well to dissolve. Adjust pH to 6.0 with 1N HCl
Add 0.5ml of Tween 20 and mix. Store at 4°C.

Vectastain Elite Rabbit IgG Kit (PK-6101)

1X PBST can be used as the diluent buffer for the blocking serum and secondary antibody. Follow the instructions written on the kit data sheet.

Evidence of positive staining:

β-catenin is localized in the hypertrophic zone of the growth plate, deeper zones of the articular cartilage, articular Chondrocytes, all the bone lining cells of the trabecular bone, as well as the cells lining the perichondrium.

Revised on 1/19/2011 by Ashish Thomas, M.S.