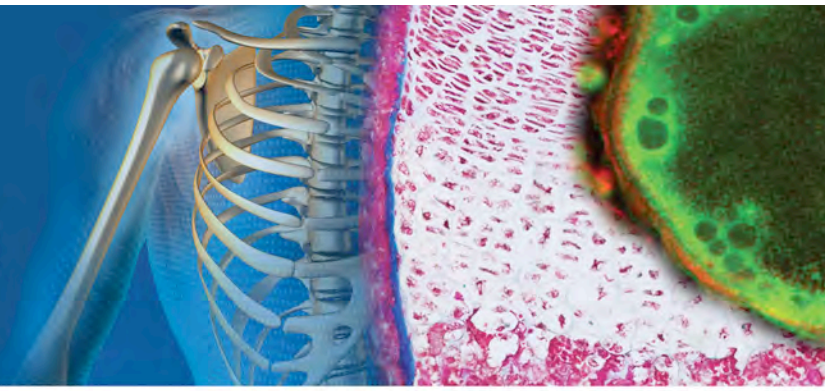

β-Catenin (Active)

Millipore cat # 05-665
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 temperature. **Set water bath to 65°C.**
Adults (2-3 month old) need a **2 hour retrieval.** Embryo sections (E18.5 & E14.5) need only a **1 hour retrieval.** After this step let the slides 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.
10. Block endogenous Avidin receptors 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. Rinse twice with 1X PBST.
14. Block nonspecific binding sites with the working solution of **Mouse Ig Blocking Reagent** *(Vector M.O.M kit # BMK-2202)* for **1 hour.**
15. Wash sections twice in PBST for 2 minutes each.
16. Incubate sections with the working solution of **M.O.M diluent** *(Vector M.O.M kit # BMK-2202)* **for 5 minutes.** Do not rinse slides after this incubation period.
17. Prepare the primary antibody in the **M.O.M diluent.** This decreases non-specific staining. Negative control slides need to be incubated with M.O.M diluent only.
18. Incubate the slides overnight **at 4° C** with a **1:100 dilution of Active β-catenin primary antibody** (*Millipore Cat# 05-665)* **for 2-3 month old** adult sections.
Use a **1:200 dilution for E18.5** and a **1:400 dilution for E14.5** embryo 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 Horse anti-mouse** secondary antibody **(***Vectastain Elite Mouse IgG Kit PK-6102)* for **30 min.**
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 the sections 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 (***Invitrogen cat # 00-8011)*
11. Wash 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 Mouse IgG Kit (PK-6102)

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:

Active β-catenin is located in the nuclei of hypertrophic chondrocytes of the growth plate, bone lining cells of the trabecular bone, as well as the cells lining the perichondrium.

Protocol standardized on mouse tissue on 6/22/2012 by Ashish Thomas, M.S.