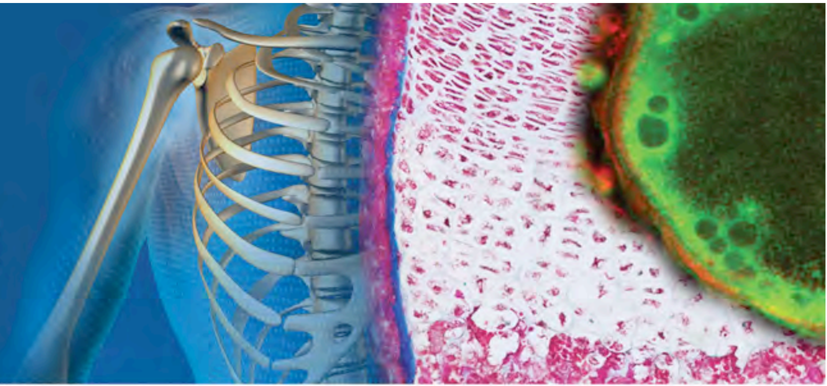

Anti –PTH/ PTHrP Receptor

(Upstate Cell Signaling Solutions Catalog # 05-517 - Mouse monoclonal antibody)

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 graded ethanols (2 changes of 100% and 95%, 1 change 70% for 5 minutes each).
3. Wash twice in deionized water for 5 minutes each.
4. Perform antigen retrieval using a **10mM Sodium Citrate Buffer pH 6.0** – This is done in a water bath at a constant temp. **Set the temp to 65°C** – leave slides in the hot water bath for **1 hour**. Then remove cover and leave outside to cool for about 10- 15 min.
5. Rinse in 3 changes of deionized water for 3-5 minutes each.
6. Outline each section with a **PAP pen.**
7. Quench endogenous peroxidase **DAKO Dual Endogenous Enzyme Blocking Reagent for 30 min (*Dako S2003*)**
8. Rinse briefly with 2 changes of PBST.
9. Block endogenous **Avidin** with the **Vector Avidin-Biotin Blocking Kit** *(Vector SP-2001)* **for 15 minutes.**
10. Rinse briefly with 2 changes of PBST.
11. Block endogenous **Biotin** with the **Vector Avidin-Biotin Blocking Kit** *(Vector SP-2001)* **for 15 minutes.**
12. Rinse briefly with 2 changes of PBST.
13. Block non-specific binding sites with the working solution of **M.O.M Mouse Ig Blocking Reagent** *(Vector M.O.M kit # BMK-2202)* prepared as described in the data sheet, for **1 hour**.
14. Wash sections twice in PBST for 2 minutes each.
15. Incubate sections in the working solution of **M.O.M diluent** *(Vector M.O.M kit # BMK-2202)* **for 5 minutes**.
16. **Do not Rinse Slides!!** Drain off M.O.M diluent and incubate **overnight at 4°C** with a **1:100 dilution of Anti-PTH/PTHrp primary antibody** *(Upstate Cell Signaling Solutions Cat # 05-517).*
17. **.** Negative Control slides need to be incubated with the M.O.M diluent only**.**

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MEDICINE of THE HIGHEST ORDER

DAY 2

1. Let slides warm up to room temperature for 15-20 minutes, then wash twice with PBST for 5 min each.
2. Incubate with the working solution of **M.O.M Anti-Mouse Secondary Antibody** *(Vector M.O.M kit # BMK-2202)* for **10 minutes.**
3. Wash twice with PBST for 2 minutes each.
4. Reconstitute the **Vectastain ABC reagent** and incubate at **room temperature for 30 min (***Vectastain Elite Mouse IgG Kit PK-6102***)**
5. Incubate with the **Vectastain ABC reagent** for **30 minutes.**
6. Wash twice with PBST, then twice in deionized water for 5 minutes each.
7. Detect color reaction with **Vector Immpact DAB** *(Vector SK-4105)* for a few minutes (check under microscope)
8. Stop the reaction with deionized water.
9. Counterstain the sections with **Hematoxylin (***Zymed Cat # 93-3943***)** for 10 minutes.
10. Wash in tap water for 5 minutes.
11. Place slides in 1X PBS for 1-3 minutes.
12. Rinse with deionized water.
13. Dehydrate through 95% ethanol (3 changes) and 2 changes of 100% ethanol.
14. Clear in 3 changes of xylene and mount with cytoseal.

Buffers:-

10mM Sodium Citrate Buffer; pH 6.0

1. Tri-Sodium Citrate Dihydrate 2.94g
2. dH2O 1000 ml

Mix well to dissolve**.** Adjust pH to 6.0 with 1N HCl
Add 0.5ml of Tween 20 and mix. Store the buffer at 4ºC for longer storage.

Evidence of Positive Staining.

Expression should be localized to the pre-hypertrophic zones of the growth plate, early hypertrophic chondrocytes, stromal osteoblast cell populations in the trabecular bone and in the deeper zones of the articular cartilage. It should **not** be expressed in the columnar cells of the growth plate.

(Revised on 10/17/2011 by Ashish Thomas)