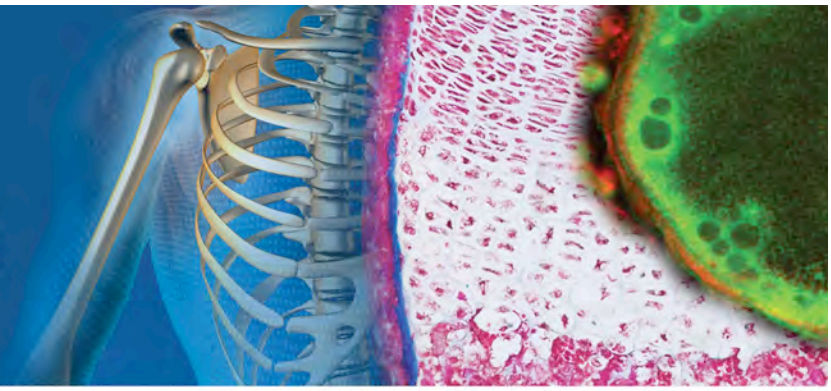

Runx2 (Cbfa1)

MBL (D130-3)
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, 5 minutes each), and rehydrate through graded alcohols (2 changes 100% and 95%, 1 change 70%, 5 minutes each).
3. Wash twice in deionized water for 5 minutes each.
4. Perform antigen retrieval using **10mM Tris EDTA Buffer pH 9.0** – This is done in a water bath at constant temp. **Set water bath temp to 65°C** and leave slides in the hot water bath for **1 hour**. Then remove cover and leave outside to cool for 10-20 minutes.
5. Rinse in 3 changes of deionized water.
6. Outline each section with a **PAP pen.**
7. Quench endogenous peroxidase in **DAKO Dual Endogenous Enzyme Blocking Reagent** (*Dako S2003*) **for 30 min**
8. Rinse with 3 changes of deionized water and once in PBST.
9. Block with **5% Normal Horse Serum.** *(Vectastain Elite Mouse IgG Kit PK-6102)*
10. Do not rinse serum off the slides.
11. Block non-specific binding sites **with the BEAT Blocking Solutions (***Invitrogen HistoMouse Plus Kit cat# 85-9541)* Add 2 drops of blocking solution **1A** to each section and **incubate for 30 min.**
12. Then rinse the sections **twice** in 1X PBST **for 3 minutes each.**
13. Add 2 drops of blocking solution **1B** to each section and **incubate for 10 min.**
14. Then rinse the sections **twice** in 1X PBST **for 3 minutes each.**
15. Finally incubate **overnight at 4°C** with a **1:100 dilution of Runx2 primary antibody** *(MBL D130-3)* **for E18.5 embryo sections or 2-3 month old adult sections.**
16. Prepare the primary antibody in **2% Normal Horse Serum**, this decreases non-specific staining.
17. Negative Control slides need to be incubated with 2% Normal Horse Serum only**.**

Day 2

1. Let the slides warm up to room temperature for 30 minutes, then wash them 5 times with 1X PBST for 5 minutes each.
2. Incubate the sections with **Vectastain Biotinylated Horse anti-mouse (***(Vectastain Elite Mouse IgG Kit PK-6102)* secondary antibody for **30 minutes.**
3. Reconstitute the **Vectastain ABC reagent** *(Vectastain Elite Mouse IgG Kit PK-6102)* and incubate at **room temperature for 30 min.**
4. Wash 5 times with 1X PBST for 5 minutes each.
5. Incubate the sections with **Vectastain ABC reagent** for **30 minutes.**
6. Wash 4 times with 1X PBST and twice in deionized water.
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 5 minutes.
10. Rinse in tap water.
11. Place the slides in 1X PBS for 1-3 minutes.
12. Rinse with deionized water.
13. Dehydrate immediately in 95% ethanol (3 changes) and 2 changes of 100% ethanol.
14. Clear in 3 changes of Xylene and mount with Cytoseal

¶ **Buffers:-**

10mM Tris-EDTA buffer (10mM Tris; 1mM EDTA; pH 9.0**)**

1. Tris Base: 2.42g
2. EDTA: 0.74g
3. Tween-20: 1.0 mL

Mix well to dissolve in 2 Liters of distilled water**.** Adjust pH to 9.0 with 1N NaOH
Store at 4ºC for longer storage.

¶ 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:-**

Expression should be localized to the hypertrophic zones of the Growth plate, sporadically present in the trabecular region. Articular Cartilage chondrocytes are negative for Runx2.

Protocol standardized on mouse tissue on 4/1/2011 by Ashish Thomas, M.S.