

Application: Immunohistochemical detection of mouse osteocalcin with Polyclonal Anti-Mouse Osteocalcin

Polyclonal Anti-Mouse Osteocalcin (Cat.# M173)

Polyclonal Anti-Mouse Osteocalcin (Cat. # M173) was used to detect osteocalcin in paraffin-embedded mouse jaw bone tissue sections.*

Protocol

- A. Tissue sections were prepared from the jaw bone of a three-week-old mouse using the following steps:
 - 1) Fixation in 4% paraformaldehyde
 - 2) Decalcification by EDTA extraction for one week at 4°C
 - 3) Dehydration with ethanol
 - 4) Penetration by xylene
 - 5) Paraffin embedding
 - 6) Sectioning of tissue
- B. Immunohistochemical staining of EDTA-decalcified paraffin-embedded mouse jaw sections with Polyclonal Anti-Mouse Osteocalcin was performed using the following method:
 - 1) Deparaffinization of the tissue sections
 - 2) Antigens were exposed by treating the sections with trypsin (25 mg/ml in 0.01 M PBS) for 20 min at 37°C
 - 3) Blocking of non-specific protein binding
 - 4) Primary antibody incubation with Polyclonal Anti-Mouse Osteocalcin (0.7 µg/ml) overnight at 4°C
 - 5) Secondary antibody exposure using the sABC System (DAKO)
 - 6) Blocking of endogenous peroxidase activity
 - 7) Incubation with streptavidin-biotin-complex for 30 min at room temperature
 - 8) Colorimetric detection of peroxidase activity using the DAB substrate
 - 9) Counterstaining with methyl green
 - 10) Dehydration, penetration and inclusion

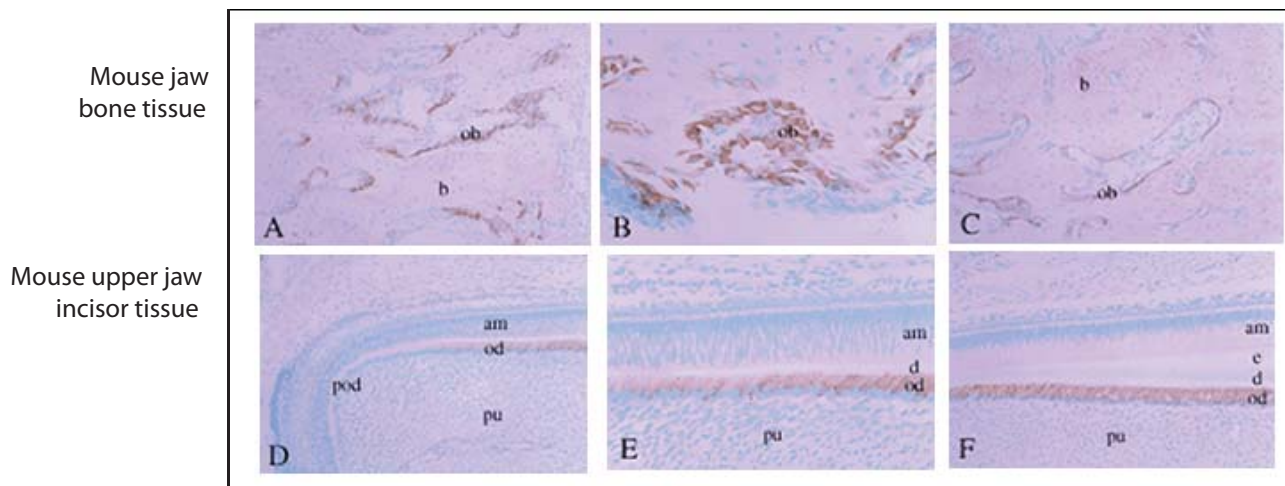
Results

- A. Strong osteocalcin signals were detected in osteoblasts (ob) along the trabecular jaw bone.
- B. Osteocalcin staining in osteoblasts (ob) shown in (A), viewed under high magnification.
- C. Osteocalcin staining in bone matrix (b).
- D. Strong positive signals were detected in odontoblasts (od) along the upper jaw incisor apical foramen, differentiated from preodontoblasts (pod).
- E. Osteocalcin staining in odontoblasts shown in (D) viewed under high magnification.
- F. Osteocalcin signals in odontoblasts at the incisal side were stronger than the staining detected in the upper jaw incisor.

continued ...

Application: Immunohistochemical detection of mouse osteocalcin with Polyclonal Anti-Mouse Osteocalcin

Polyclonal Anti-Mouse Osteocalcin (Cat.# M173)



Abbreviations: b, bone; ob, osteoblast; pod, preodontoblast; d, dentin; od, odontoblast; e, enamel; am, ameloblast; pu, pulp

*This experiment was performed by Prof. Satoshi Toyosawa, Department of Oral Pathology, Graduate School of Dentistry, Osaka University.