Mouse Anti-Human Bone Sialoprotein, (BSP) monoclonal

CatNo MAK5209G

BatchNo: See Label
Expiration Date: See Label
Storage: 2-8°C lyophilised
After Reconstitution: -20°C

Introduction:	Bone sialoprotein (BSP) is a phosphorylated and sulphated glycoprotein that is expressed by mineralized connective tissues. It constitutes 8-12% of the total non-collagenous proteins in bone and cementum. BSP and related glycoproteins such as osteonectin and osteopontin play a regulatory role in bone metabolism, e.g. they control bone mineralisation and resorption. The serum concentration of BSP therefore reflects bone remodeling processes. BSP is also produced in tumors that frequently metastasize to bone, such as breast, prostate and thyroid cancer.
Synonyms:	Bone sialoprotein 2, BSP2, Cell-binding sialoprotein, Integrin-binding sialoprotein
Swiss-Prot No:	P21815
Gene Information:	Gene name: IBSP GeneID: 3381
Clone Number:	ID1.2
Volume/Quantity:	0.1 mg
Reconstitution:	Reconstitute with 0.1 ml distilled water
Product Form:	Purified IgG - lyophilised
Preparation:	Purified IgG prepared by affinity chromatography on Protein G from tissue culture supernatant
Buffer:	50 mM Tris pH7,4
Approx. Protein Concentrations:	IgG concentration 1.0 mg/ml
Immunogen:	human Bone Sialoprotein (bone extract)
Isotype:	IgG1 (Mouse)
Specificity:	Human Bone Sialoprotein, recombinant h BSP, recombinant porcine BSP and recombinant rat BSP. There were no cross reactivities obtained with human Osteonectin, human Osteopontin, native porcine BSP, porcine OPN, porcine ON and native rat BSP.
Species Cross Reactivity:	Reacts with: Rabbit **N.B.** Antibody reactivity and working conditions may vary between species.

Applications:

		Suggested Working Dilution
FlowCytometry	Yes	
Immunohistology-frozen	Yes	
Immunohistology-paraffin	Yes	1/2000
Immunohistology-resin	Yes	
ELISA	Yes	
Immunoprecipitation	Not tested	
Western Blotting	Yes	1/1000 – 1/4000
Radioimmunoassay	Yes	

Where this antibody has not been tested for use in a particular technique this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. It is recommended that the user titrates the antibody for use in their own system using appropriate negative/positive controls.

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Characteristics:

Normal tissues:
In paraffin sections the antibody labels osteoblasts and osteoid osteocytes in a paranuclear staining; osteoclasts display staining at the cell/bone interface and at multiple perinculear rings and cytoplasmic dots. Staining of the bone matrix is intense but uneven. No labelling was observed in preosteogenic cells in the inner layer or fibroblastic cells in the outer layer of the periostium. In addition the antibody labels hypertrophic chondrocytes in the growth plate with a cytoplasmic staining; cartilage matrix remained completely unstained in undigested sections, but was faintly positive in sections predigested with Chondroitin ABC lyase. The antibody labels the cell surface of syncytial trophoblast and cytotrophoblast cells of the placental villi. The antibody does not label ocular tissues, skin, tendon, muscle, or kidney.

Abnormal tissues:
In paraffin sections the antibody labels the cytoplasm of mammary, prostate, thyroid cancerous cells as well as bone metastases and subcutaneous tumors formed by MDA-MB-231 cells in nude mice. The antibody labels squamous lung carcinoma and lung adenocarcinoma with a cytoplasmic and membrane-associated pattern, but not bronchioloalveolar cancers. In addition the antibody labels high grade squamous intraepithelial lesions and invasive squamous cell carcinoma of the uterine cervix.

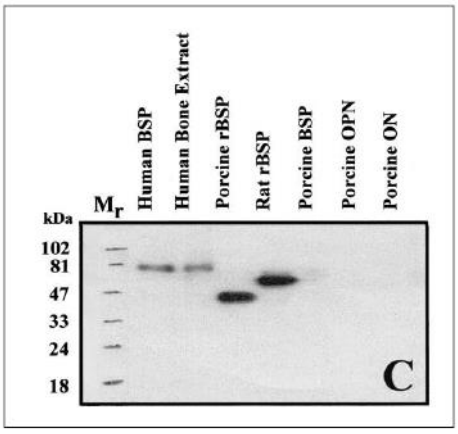

Figure 1:
Immuno Blot analysis of MAK5209 specificity. Native and recombinant bone proteins were separated by SDS-PAGE and immunoblotting with MAK5209 (1:4000). MAK5209 recognizes human and recombinant BSP but not porcine native BSP, porcine Osteopontin (OPG) or SPARC/Osteonectin (ON).

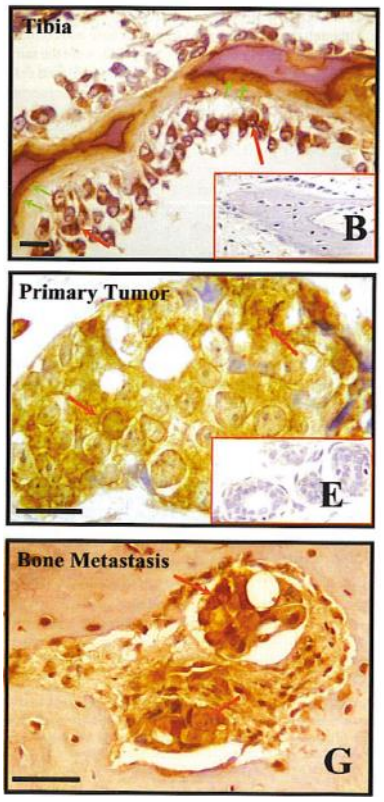

Figure 2:
Immunohistochemistry image of BSP staining in paraffin sections of human tissue. Sections were incubated with MAK5209 (1:2000) and detected using Biotin-conjugated secondary antibody and Vectastain *Elite* ABC Kit. DAB was used as the chromogen. The sections were counterstained with Harris' hematoxylin. Sections of **B**. embryonic tibia; **E**. primary breast carcinoma; and **G**. bone metastasis from the same patient were stained with MAK5209. Inserted within B and E are control sections incubated with mouse IgG. Scale bar = 20µm.

Cogan G. et al. (2004) Connect Tissue Res 45(1):60-71

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Immunohistology

Recommended Secondary Reagents:	F(ab')$_2$ rabbit anti-mouse IgG HRP conjugate - (LINARIS CatNo LST0013B) **Peroxidase** Vectastain® ABC-*Elite* Mouse IgG (Vector CatNo PK-6102) DAB-Substrate (LINARIS CatNo E108) or HistoGreen-Substrate (LINARIS CatNo E109) **Alkaline Phosphatase** Vectastain® ABC Mouse IgG (Vector CatNo AK-5002) Vector® Red (Vector CatNo SK-5100).
Recommended Negative Controls:	Mouse IgG1 Negative Control (LINARIS CatNo ITC0928)

Westernblotting

Chemiluminescent Substrate:	DuoLuX Chemiluminescent Substrate for Alkaline Phosphatase (Vector CatNo SK-6605) or Peroxidase (Vector CatNo SK-6604).
Western Blotting Immunodetection Kit:	Vectastain ABC-AmP Chromogenic Detection Kit (BCIP/NPT Substrate) for Mouse IgG (Vector CatNo AK-6402) or forRabbit IgG (Vector CatNo AK-6401)
Weight Standard:	Biotinylated protein molecular weight marker (Vector CatNo SP-1400). Molecular weight marker from 19kD to 222kD.

References

1. Wuttke M, Muller S, Nitsche DP, Paulsson M, Hanisch FG, Maurer P (2001). Structural characterization of human recombinant and bone-derived bone sialoprotein. Functional implications for cell attachment and hydroxyapatite binding. J Biol Chem 276(39): 36839-36848.
2. Ibrahim T, Leong I, Sanchez-Sweatman O, Khokha R, Sodek J, Tenenbaum HC, Ganss B, Cheifetz S (2000). Expression of bone sialoprotein and osteopontin in breast cancer bone metastases. Clin Exp Metastasis 18(3): 253-260.
3. Cogan G, Bansal AK, Ibrahim S, Zhu B, Goldberg HA, Ganss B, Cheifetz S, Armbruster FP, Sodek J (2004). Analysis of Human Bone Sialoprotein in Normal and Pathological Tissues using a Monoclonal Antibody (BSP 1.2 mab). Connective Tissue Research 45(1): 60 – 71.
4. Hilbig H, Kirsten M, Rupietta R, Graf HL, Thalhammer S, Strasser S, Armbruster FP (2007). Implant surface coatings with bone sialoprotein, collagen, and fibronectin and their effects on cells derived from human maxillar bone. Eur J Med Res 12(1): 6-12.
5. Scherberich A, Galli R, Jaquiery C, Farhadi J, Martin I (2007). Three-dimensional perfusion culture of human adipose tissue-derived endothelial and osteoblastic progenitors generates osteogenic constructs with intrinsic vascularization capacity. Stem Cells 25(7): 1823-1829.
6. Tobias Bäuerle, Hassan Adwan, Fabian Kiessling, Heidegard Hilbig, Franz P. Armbruster and Martin R. Berger (2005). Characterization of a rat model with site-specific bone metastasis induced by MDA-MB-231 breast cancer cells and its application to the effects of an antibody against bone sialoprotein. Int. J. Cancer: 115, 177–186
7. Graf HL, Bruecker M, Troeger U, Hilbig H (2012). Proliferation, Apoptosis and Expression of Non-Collagenous Proteins: Differences between the Upper and the Lower Jaw Bone in vitro. Cells Tissues organs 195 (3): 244-251

Storage Conditions:	Prior to reconstitution store at 2-8°C. After reconstitution store at -20°C Avoid repeated freezing and thawing as this may denature the antibody. Should this product contain a precipitate we recommend microcentrifugation before use.
Shelf Life:	12 months from date of reconstitution.
Health and Safety Information:	(A full Health and Safety assessment is available upon request)

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