

ProductInformation

Monoclonal Anti-Human Placental Alkaline Phosphatase Clone 8B6

Mouse Ascites Fluid

Product Number **A 2951**

Product Description

Monoclonal Anti-Human Placental Alkaline Phosphatase (mouse IgG2a isotype) is derived from the 8B6 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from immunized BALB/c mice. Whole human epidermoid carcinoma cell line expressing placental alkaline phosphatase was used as the immunogen.¹ The isotype is determined using the Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay, using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Human Placental Alkaline Phosphatase reacts with both Regan and Nagao isozymes of human placental alkaline phosphatase (hPLAP, 130 kDa, 67/130 kDa in SDS gels).¹ The antibody binds to hPLAP with an affinity constant of $5 \times 10^9 \text{ LM}^{-1}$ when used in RIA.¹ It does not react with PLAP-like enzymes. Using immunohistochemical techniques, the antibody stains cultured cells, frozen and formalin-fixed, paraffin-embedded sections. The product may be used in ELISA (using whole cell or capture assay),¹ for the radioimmunolocalization of xenografts of human cell lines expressing PLAP (e.g. in nude mice),² and in immunoblotting.

Alkaline Phosphatase (AP) is a broad and general term associated with non-specific phosphomonoesterases, having optimal activity at alkaline pH. Alkaline phosphatase in extracts of human tissues and in serum displays considerable heterogeneity with respect to net molecular charge, size, and antigenic distinction.³ Recognition of the nature and occurrence of these multiple forms has made a significant contribution to the understanding of changes in alkaline phosphatase serum concentrations in disease and the use of alkaline phosphatase measurements in diagnosis.⁴

Human placental alkaline phosphatase (EC 3.1.3.1; hPLAP), an isoenzyme of the AP group of enzymes, is ordinarily synthesized in the placental syncytiotrophoblast, becoming detectable in the maternal circulation after the twelfth week of pregnancy.⁴ Small amounts of hPLAP are found in the endocervix, Fallopian tubes and lung. Very small amounts of heat-stable AP resembling hPLAP (hPLAP-like AP) are expressed in the testis, thymus and in rare colon epithelial cells. Placental alkaline phosphatase differs from the alkaline phosphatase of bone, liver and kidney, by the relative rate of hydrolysis of various orthophosphate and pyrophosphate substrates, and in its greater degree of inhibition by L-phenylalanine and lower inhibition by L-homoarginine or levamisole.

These characteristics of placental phosphatase are shared by the alkaline phosphatase of the small intestine, indicating a considerable similarity between the substrate- and ligand-binding sites of the two isoenzymes.⁴ Human alkaline phosphatase constitutes a system of multiple molecular forms of enzymes in which heterogeneity is partly due to genetic factors and partly to post-translational modifications, such as the modulation of enzyme protein production by inducers such as corticosteroids, butyrate and sodium chloride. A glycopospholipid group enables anchoring of the mature PLAP to membranes by covalent linkage to components such as ethanolamine and other carbohydrate groups.

The allelozymes of placental phosphatase have characteristic electrophoretic mobilities under defined conditions. A dimeric structure of placental phosphatase has been inferred from the presence of three-membered sets of isoenzymes in placental extracts from subjects who are heterozygous at the placental phosphatase locus. The discovery of the

"Regan" AP isoenzyme, with the properties of hPLAP, in the serum of a patient with terminal bronchogenic cancer initiated the interest in this antigen as an oncodevelopmental marker. Since then, the presence of hPLAP and hPLAP-like AP has been described in sera and tumor tissues of patients suffering from various types of cancer. The highest frequency of ectopic hPLAP expression was found in germ-cell tumors (especially testicular seminomas) and ovarian cancers. The discovery that some forms of hPLAP are synthesized ectopically by some malignant tumors indicates that its presence may be a criterion for localizing cancer cells. Non-epithelial tumors rarely exhibit hPLAP immunoreactivity.

Despite the continued interest in hPLAP as a potential tumor marker, it has not been widely used in the routine clinical laboratory because of the low overall specificity attained by such methodologies as heat-inactivation and sensitivity to L-phenylalanine. The use of polyclonal anti-hPLAP antibodies was hampered by the cross-reactivity of these antibodies with the common epitopes of intestinal AP. Reproducible and accurate immunological quantification of hPLAP has become possible with the advent of hPLAP-specific monoclonal antibodies. The use of monoclonal antibodies as reagents has greatly improved the sensitivity of the tests used to distinguish placental isozymes from their tumor cell counterparts, offering the hope of realizing the diagnostic potential of the hPLAPs.

Monoclonal Anti-Human Placental Alkaline Phosphatase may be used for the localization of hPLAP using various immunochemical assays such as ELISA (using whole cells or capture assay), immunoblot, immunocytochemistry, and radio-immunolocalization.

Reagents

The product is provided as ascites fluid with 15 mM sodium azide as a preservative.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

The minimum antibody titer of 1:4,000 was determined by immunoperoxidase staining of formalin-fixed, paraffin-embedded, sections of human placenta.

In order to obtain best results in different techniques and preparations, it is recommended that each individual user determine their optimum working dilutions by titration assay.

References

1. Durbin, H., et al., *Int. J. Cancer Suppl.*, **2**, 50 (1988).
2. Durbin, H., et al., *Int. J. Cancer Suppl.*, **2**, 59 (1988).
3. Stigbrand, T., and Fishman, W., eds., *Human Alkaline Phosphatases*, p. 66, (Prog. Clin. Biol. Res., Alan R. Liss, New York, 1988).
4. Moss, D. W., *Clin. Chem.*, **28**, 2007 (1982).

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