Product No. B-9277
Lot 017H4856

Monoclonal Anti-Human Band 3
Mouse Ascites Fluid
Clone BIII-136

Monoclonal Anti-Human Band 3 (mouse IgG2a isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Glycophorin B purified from human erythrocytes was used as the immunogen. The isotype is determined using Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is provided as ascites fluid with 0.1% sodium azide (see MSDS)* as a preservative.

Specificity

The antibody recognizes an epitope located in the cytoplasmic pole of the Band 3 molecule. This epitope is within approximately 20 kD from the N-terminal end. This antibody recognizes Band 3 protein (90-100 kD) and several lower molecular mass peptides migrating in SDS-PAGE gels in the regions of 60, 40 and 20 kD. Digestion and aggregation of the Band 3 protein results in its detection with multiple molecular weight proteins. Since the epitope is not located at the erythrocyte surface, the antibody does not agglutinate red blood cells and its binding to the cell surface cannot be detected by an indirect agglutination assay. Monoclonal Anti-Human Band 3 does not localize Band 3 from horse, bovine, pig, guinea pig, dog or mouse erythrocytes, nor with human fibroblast extract (non-erythroid). This antibody was found to be specific for the cytoplasmic amino-terminal protein of band 3.[1]

Description

The Band 3 protein is the most abundant integral protein of human erythrocyte membranes and functions as the major anion transporter polypeptide of erythrocytes. Band 3 is a 90-100 kD protein, its micro-heterogeneity is ascribed mainly to the structural heterogeneity of the carbohydrate moiety. The C-terminal portion of the molecule spans the membrane several times, and is responsible for anion transport. It carries only large N-linked oligosaccharide chain of the polylactosamine type, located outside the red cell. The N-terminal portion, comprising almost half of the polypeptide chain of Band 3, is located inside the red cell and interacts with cytoskeletal and cytoplasmic proteins such as ankyrin (Band 2.1), Bands 4.1 and 4.2, hemoglobin and some glycolytic enzymes. Band 3 is a highly hydrophobic protein, it exists in erythrocytes as a dimer and tetramer and has a strong tendency to aggregate as a result of the oxidative stress. Aggregation can be avoided if the membranes are solubilized in the presence of N-ethylmaleimide.

Three other groups of bands localized by this antibody are in the regions of 55-60 kD, 38-42 kD, and 21-26 kD, which for the sake of simplicity are called 60 kD, 40 kD and 20 kD peptides res-pectively.[2] The 20 and 40 kD fragments are formed by cleavage on the cytoplasmic side. Even though the 60 kD fragment is transmembrane protein, its cleavage site is available at the external surface of the erythrocyte.

Proteins similar to human erythrocyte Band 3 are present in animal erythrocytes and, in small amount, in non-erythroid cells with a high degree of homology in the transmembrane domains and considerably lower homology in the cytoplasmic domains of the protein. Band 3 protein is degraded to a small extent *in vivo* and is relatively stable in intact erythrocytes, but its degradation proceeds quickly after hemolysis and during preparation of the membranes, even if it is performed in the cold in the presence of protease inhibitors. An increase in the amount of all degradation products of Band 3 was distinctly correlated with the decrease of creatine content, i.e. with erythrocyte age. Senescent cell antigen, an aging protein that terminates the life-span of cells by initiating IgG binding and cellular removal appears to be derived from Band 3.

Uses

Monoclonal Anti-Human Band 3 may be used in the study of red cell structures and functions and to study the fragmentation of the cytoplasmic domain of band 3 protein *in vivo* and *in vitro*. The antibody may be used in immunoblotting, immunoprecipitation, ELISA and for immunofluorescent staining of fixed human erythrocytes.

Titer: 1:5,000

The antibody titer was determined by indirect immunoblotting using an extract of human erythrocyte ghosts.

In order to obtain best results it is recommended that each individual user determine their working dilution by titration assay.

References

1. Rouger, P. and Anstee, D., Vox Sanguis, **55**, 57 (1988).
2. Czerwinski, M., et al., Eur. J. Biochem., **174**, 647 (1988).

Storage

For continuous use, store at 2-8°C. For extended storage, the solution may be frozen 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.

*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 hazards and safe handling practices.