3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

MONOCLONAL ANTI-DESMOSOMAL PROTEIN
Clone ZK-31
Mouse Ascites Fluid

Product Number **D 1286**

Product Description

Monoclonal Anti-Desmosomal Protein (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Human epidermal keratins were used as the immunogen. The isotype is determined using 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-Desmosomal Protein is immunospecific for an epitope on desmosomes as determined the indirect immunofluorescent staining of frozen sections of human and animal tissues. It may also be used for immunoperoxidase staining of frozen sections of human and animal tissues or densely grown monolayers of human and animal cells fixed with methanol (–20 °C, 10 min) where it stains the cell-cell boundaries not stained by anti-cytokeratin antibodies.[1] Monoclonal Anti-Desmosomal Protein shows no staining of cytoplasmic filaments and it displays the typically punctate dot-like pattern consistent with desmosome decoration. The product does not stain hemidesmosomes. This antibody shows positive staining with human mammilla (epidermis and ducts), tonsil, cervix and kidney, bovine muzzle and salivary glands, mouse liver (hepatocytes and bile duct epithelia) and snout, rabbit tongue, rat tongue, lip and heart. The antibody localizes desmosomes on MCF7, BMGE, and PtK2 monolayers grown in tissue culture. Weak reactions are observed with SDS-denatured and reduced desmosomal protein preparations by immunoblotting.[1] Fixation with formalin seems to destroy the epitope recognized by this antibody. The antibody may aid in the differentiation of tumors of epithelial and other origin and especially for tumors which show poor morphological differentiation.

Several types of cell to cell interconnections or junctions are known and have been morphologically characterized tight junctions, desmosomes, adhesion plaques and gap junctions. Desmosomes (zonulae adherence, adherence junctions) are thickened regions of the plasma membrane where cells are tightly attached to their neighbors. Hemidesmosomes are similar structures found in regions of epithelial cells in contact with the basal lamina. Desmosomes and hemidesmosomes are specifically associated with intermediate sized filaments of the cytokeratin type.

The histological determination of tissue type has been facilitated in recent years by immunological techniques which use specific antibodies directed against various tissue markers such as intermediate filament proteins.

Reagents

Monoclonal Anti-Desmosomal Protein is provided as ascites fluid with 0.1% sodium azide.

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 °C to 8 °C for up to one month. For extended storage, solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify by centrifugation before use.

Product Profile

A minimum working dilution of 1:400 was determined by indirect immunofluorescent staining of frozen sections of human tissue.

In order to obtain optimum results, it is recommended that each individual user determine working dilutions by titration assay.

Procedure

Indirect Immunofluorescent Labeling of Frozen Tissue Sections

1. Pre-cool isopentane in liquid nitrogen. Dip freshly dissected tissue blocks (5 x 5 mm pieces) quickly into cold isopentane. **Aldehyde fixation should be avoided**. Frozen tissue blocks may be stored in sealed vials at –70 °C, in the presence of a few drops of isopentane to prevent drying.
2. Transfer frozen tissue blocks to the chamber of the cryostat and allow temperature to equilibrate for about 30 minutes.
3. With the embedding medium, mount the tissue block on the stub.
4. Trim the surface of the block at –20 °C or temperature found optimal for the particular tissue type.
5. Cut 3-5 micron sections. Transfer sections to clean glass microscope slides and allow to dry at room temperature (25 °C) for 5-16 hours.
6. Immerse the slides in the pre-cooled acetone (–20 °C) and leave for 20 minutes.
7. Allow slides to dry briefly at room temperature.
8. Immerse slides for 10 minutes in staining jar filled with PBS (phosphate buffered saline) at room temperature.
9. Dilute monoclonal antibody to its optimal dilution with PBS.
10. Lay slides flat, section-side up, in humid chamber. Pipette 50-70 µl of diluted monoclonal antibody over sections on slide. Cover chamber and leave slides undisturbed at room temperature or at 37 °C. Incubation at 37 °C increases sensitivity without increasing background staining.
11. Transfer slides to staining jar filled with PBS at room temperature. Replace PBS at least twice at 10 minute intervals.
12. Dilute second antibody conjugate with PBS.
13. With a tissue, wipe slides dry **around** the sections. **Do not touch sections**. Lay slides flat in humid chamber. Pipette 50-70 µl diluted second antibody over each section. Cover chamber and leave slides undisturbed at 37 °C for 30 minutes.
14. Wash slides 3 times in PBS.
15. Place a drop of 90 % glycerol or other aqueous mounting medium over the sections and slowly lower coverslip into place, avoiding bubbles. Coverslip may be sealed around the edges with clear nail polish.
16. Examine with a fluorescence microscope.

References

1. Lang, A.B., et al., Exp. Cell Biol., **54,** 61-72 (1986).

AC 11/6/00