Product Information

Monoclonal Anti-Desmin, Clone DE-U-10

Produced in Mouse, Ascites Fluid

D1033

Product Description

Monoclonal Anti-Desmin (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Desmin purified from pig stomach was used as the immunogen. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Cat. No. ISO2.

Monoclonal Anti-Desmin is immunospecific for desmin as determined by indirect immunofluorescent staining methods. Monoclonal Anti-Desmin specifically stains the wide desmin band of 50-55 kDa by immunoblotting methods. The antibody reacts specifically with desmin in cultured cells or tissue preparations from cat, bovine, goat, hamster, human, mouse, pig, rabbit, rat, sheep, chicken, lizard, and viper.

Monoclonal Anti-Desmin may be used for immunocytochemical localization of intermediate filaments of the desmin group in all types of muscle cells and to localize desmin at the periphery of z-discs. The product also specifically stains desmin when used in immunoblotting assays. Monoclonal Anti-Desmin labels desmin in tumors derived from muscle tissue (leiomyomas and rhabdomyosarcomas).[1]

Desmin is the protein subunit of muscle-type intermediate filaments. Intermediate filaments (IFs), with characteristic 10 nm diameter are a distinct class of heterogenous protein subunits apparent by both immunological and biochemical criteria. IFs differ significantly from the other cytoskeletal elements of the cell, namely microtubules and microfilaments, and are components of most eukaryotic cells. Desmin is one of the five major groups of IFs and is found predominantly in skeletal, cardiac, and smooth muscle.

Reagent

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

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for a maximum of one month. For extended storage, solution may be frozen in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify by centrifugation before use.

Procedure

Indirect Immunofluorescence Labeling of formalin-fixed, paraffin-embedded tissue with Monoclonal Anti-Desmin.

Materials

- Paraffin sections (4-6 µm) of animal or human tissue (material fixed with 10% phosphate buffered formalin).
- Phosphate buffered saline (PBS), pH 7.4, containing 1% BSA, Cat. No. P3688.
- Protease, Cat. No. P5147, 0.1% (w/v) in PBS, Cat. No. P4417.
- Primary antibody: Monoclonal Anti-Desmin.
- Secondary antibody: Anti-Mouse IgG (Fab specific)-FITC, Cat. No. F5262, at appropriate dilution in PBS.
- Mounting medium.
- Isotype Control: Mouse IgG1, kappa ascites fluid, Cat. No. M7894, or Control Ascites Fluid, Cat. No. M8273.

Method

Standard Deparaffinization Procedure

Immediately before staining:

1. Pre-heat sections at 56 °C for 1 hour
2. Immerse in xylene bath for 5 minutes at room temperature (RT). Repeat with fresh xylene.
3. Rehydrate in 95% ethanol for 2 minutes at room temperature. Repeat with fresh 95% ethanol.
4. Rehydrate in PBS for 5 minutes at room temperature.
   Note: Immersions are carried out with occasional agitations.

Enzymatic Unmasking of Antigenic Sites

1. Drain PBS and quickly proceed to next step.
2. Treat section with 2 drops (approx. 100 µL) of Pronase solution for 15 minutes (RT).
3. Wash thoroughly in PBS (preferably in the cold) for 5 minutes to remove residual enzyme and quickly proceed to next step.

Test

1. Apply 2 drops of Monoclonal Anti-Desmin diluted appropriately in PBS containing 1% BSA.
2. Incubate for 1 hr. at 37 °C.
3. Gently rinse in PBS for 5 mins. Repeat with fresh PBS.
4. Drain, carefully blot away excess moisture around the sections.
5. Quickly apply freshly prepared diluted FITC secondary antibody (at previously determined appropriate dilution - usually 1:20-1:40) in PBS containing 1% BSA.
6. Incubate for 30 mins at 37 °C.
7. Gently rinse in PBS for 5 mins. Repeat with fresh PBS. Blot away excess moisture from around the sections.
8. Add mounting medium and coverslip.
9. Examine section using a UV fluorescent microscope. Mounted preparations can be stored in a refrigerator.

NOTE: Do not allow tissue sections to dry out at any time during the procedure.

In case of excessive background staining remove aggregates from antibodies by centrifuging for 15 min immediately prior to use.

Product Profile

Indirect immunofluorescence

A minimum working dilution of 1:40 was determined using formalin-fixed, paraffin-embedded human tissue sections.

Immunoblotting

A minimum working dilution of 1:200 was determined using chicken gizzard extract/whole extract of mouse myoblast C2C12 cells.

Note: In order to obtain best results, it is recommended that each individual user determine their optimal working dilutions by titration assay.

References

1. Debus, E., et al., *EMBO J.*, **2**, 2305 (1983).

Notice

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