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Product Information

Monoclonal Anti-Biotin Cy3™, clone BN-34
produced in mouse, purified immunoglobulin

Catalog Number **C5585**

Product Description

Monoclonal Anti-Biotin (mouse IgG1 isotype) is derived from the BN-34 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Biotinylated KLH was used as the immunogen. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. The product is prepared by conjugation of Cy3[1] to Protein A purified Monoclonal Anti-Biotin antibody. The conjugate is purified by gel filtration to remove unbound Cy3.

Monoclonal Anti-Biotin recognizes the free biotin molecule by competitive ELISA. Specificity is verified using biotinylated goat antibodies reactive with human and rabbit antigens coated on microtiter plates. Monoclonal Anti-Biotin- Cy3 recognizes biotinylated polyclonal and monoclonal immunoglobulins in indirect immunohistology.

Biotin is a naturally occurring vitamin and co-enzyme required by cells in living organisms or in culture. Usually biotin can be easily coupled to antibodies without disturbing the biological properties of the antibody. Conjugation of the biotin moiety to purified antibodies may be used to identify biomolecules in fluorescence microscopy. Biotin labeled antibodies can be detected using avidin or streptavidin coupled to fluorochromes. Alternatively, detection of the biotin hapten is possible by use of a specific labeled fluorochrome conjugate of anti-biotin. The fluorescent signal produced by Cy3 antibody conjugates is highly intense and exhibits minimal non-specific binding, aggregation or quenching properties. In addition, Monoclonal Anti-Biotin- Cy3 can be used in many other applications where biotin can be introduced as target label. It can be used in detection of DNA and mRNA by sensitive non-isotopic *in situ* hybridization. It may also be used for detection of microinjected biotin-haptenized cytoskeletal proteins allowing examinination of the pattern of incorporation and turnover of cytoskeletal proteins in living cells. The conjugate may also be used in the study of glycoconjugates in conjunction with biotinylated lectins, and for detecting biotinylated derivatives of peptide hormones used as probes for the localization of membrane receptors in cells by immunocytochemical techniques.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at 2-8 °C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

Indirect Immunofluorescent Staining of Tissue Sections

Materials

1. Formalin-fixed, paraffin-embedded sections (4-6 µm) of animal or human tissue.
2. PBS, pH 7.4, Catalog Number P3813.
3. PBS, pH 7.4 containing 1% BSA, Catalog Number P3688, diluent.
4. Xylene (xylol)
5. Ethanol 95%, Ethanol 80%, Ethanol 70% in H_2O
6. Biotinylated antibody, at recommended dilution.
7. Monoclonal Anti-Biotin- Cy3
8. Mounting medium

Standard deparaffinization procedure

1. Place the slides in a 56-60 °C oven for 15 minutes.
2. Clear in two changes of xylene for 5 minutes each.
3. Shake off excess liquid and rehydrate slides in two changes of fresh absolute ethanol for 3 minutes each.

4. Shake off excess liquid and place slides in fresh 90% ethanol for 3 minutes.
5. Shake off excess liquid and place slides in fresh 80% ethanol for 3 minutes.
6. Rinse the slides in gently running tap water for 30 seconds. (Avoid a direct jet which may wash off or loosen the section). Place in PBS wash bath for 30 min. at room temp. for further rehydration

Immersions are carried out with occasional agitations. In some cases, enzymatic pre-treatment of the tissue may be necessary to unmask antigen. Use 0.1% trypsin, Catalog Number T7168, for 15 minutes at 37 °C.

Staining
1. Apply 100 µL of the diluted biotinylated antibody in PBS containing 1% BSA.
2. Incubate for 1 hr at room temperature or at 37 °C.
3. Gently rinse twice in PBS for 5 minutes each.
4. Apply 100 µL of the diluted Monoclonal Anti-Biotin-Cy3 in PBS containing 1% BSA.
5. Incubate for 1 hr at room temperature or at 37 °C.
6. Gently rinse twice in PBS for 5 minutes each, drain.
7. Carefully blot excess moisture around sections.
8. Add mounting medium and coverslip.
9. Observe using UV fluorescent microscope. Mounted preparations can be stored in a refrigerator.

Indirect Staining of Cultured Cells

Materials
1. Coverslips.
2. Cells in DMEM medium + 10% fetal calf serum.
3. 10 mM PBS, pH 7.4, Catalog Number P3813, without preservative.
4. PBS containing 1% BSA, Catalog Number P3688, diluent
5. Absolute methanol, cooled at –20 °C.
6. Acetone, Analytical grade, cooled at –20 °C
7. Aqueous mounting medium
8. Primary unlabeled antibody and/or biotinylated antibody, at recommended dilutions.
9. Secondary biotinylated antibody, at recommended dilution.
10. Monoclonal Anti-Biotin- Cy3

Cell Growth and Fixation
1. Collect cells from tissue culture dish at a stage of almost confluency, wash with medium and seed onto coverslips. Seed 1-2 x 10^4 cells per coverslip and grow cells in incubator for 2-3 days. Do not change medium.
2. Remove coverslips from incubator, aspirate medium.
3. Wash twice with PBS, remove solution by aspiration.
4. Add enough cold methanol to cover the cell layer. Incubate for 10 minutes at –20 °C. Aspirate solution.
5. Rinse cell layer twice for 10 sec. with cold acetone,
6. Aspirate, wash twice with PBS. Rehydrate in PBS for at least 30 minutes prior to labeling with antibody.

Staining
1. Dilute primary unlabeled antibody or biotinylated antibody in PBS containing 1% BSA. Add enough diluted antibody to cover the cell layer and incubate coverslips for 60 minutes at room temperature.
2. Wash 3 times with PBS, 5 minutes each.
3. If unlabeled primary antibody was used, apply 100 µL of the diluted biotinylated secondary antibody and incubate for 30 minutes at room temperature.
4. Wash 3 times with PBS, 5 minutes each.
5. Apply 100 µL of the diluted Monoclonal Anti-Biotin-Cy3 and incubate for 30 minutes at room temperature.
6. Wash 3 times with PBS, 5 minutes each.
7. Drain excess solution by touching edge of coverslip on paper toweling. Invert coverslips onto mounting media applied on glass slides.
8. Read under the UV fluorescent microscope. Mounted preparations can be stored in the dark at 2-8 °C.

Notes
1. Do not allow tissue sections or cell layers to dry out at any time.
2. In case of excessive background staining remove aggregates from the labeled reagent (conjugate) by centrifuging for 15 minutes immediately prior to use.

Product Profile

Immunohistochemistry: a dilution of at least 1:400 was determined by staining of formalin-fixed, paraffin-embedded tissue sections.

Indirect Immunofluorescence: a dilution of at least 1:200 was determined by staining of cultured cells.

Note: In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

References

1. Southwick, P. L., et al., *Cytometry*, **11**, 418 (1990).

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