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ProductInformation

Monoclonal Anti-MAT™
Clone MAT1-87
Purified Mouse Immunoglobulin

Product Number **M 6693**

Product Description

Monoclonal Anti-MAT™ (mouse IgG2a isotype) is derived from the MAT1-87 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic MAT peptide HNHRHKHGGGC conjugated to KLH.

Monoclonal Anti-MAT recognizes the seven amino acid MAT epitope sequence within either N or C terminal MAT-tagged fusion proteins Applications for this antibody include ELISA, immunoblotting, immunoprecipitation and immunocytochemistry.

Recombinant DNA technology enables the attachment of specific sequences to genes of interest to provide "affinity handles" (tags) designed to enable the selective identification and purification of the protein of interest.[1-6] The addition of a tag to a given gene creates a stable fusion product that may not interfere with the bioactivity of the protein, or with the biodistribution of the tagged product.

The MAT tag is a seven amino acid peptide tag that binds to transition metals such as Nickel and Cobalt. This tag, which consists of the sequence HNHRHKH, allows the purification of MAT fusion proteins using metal-based affinity chromatography such as the highly selective HIS-Select™ Nickel Affinity Gel (Product No. P 6611). The MAT tag sequence can be incorporated into both prokaryotic and eukaryotic expression vectors. A variety of expression vectors are available from Sigma-Aldrich (Product No. E 5530, E 5780, E 5280, C 5864, T 6699 and many more).

Monoclonal antibodies reacting specifically with the MAT tag may be useful in various immuno detection techniques to identify the expression of a MAT fusion protein or to aid in isolation and purification when MAT-fusion proteins are produced in prokaryotic or eukaryotic host cells.

Reagent

The product is provided as a solution in 0.01M phosphate buffered saline, pH 7.4, containing
15 mM sodium azide as a preservative.

Antibody concentration: approx. 2 mg/ml.

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. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A working concentration of 1- 2 µg/ml is determined by immunoblotting, using cell extracts expressing a C-terminal MAT-tagged protein.

5-10 µg of the antibody can immunoprecipitate a recombinant C-terminal MAT-tagged protein.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilution by titration test.

Procedures

Immunoblotting

All incubation steps should be performed at room temperature

1. Separate MAT- tagged proteins from sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 10-20 µg total lysate protein per lane. Note: The amount of lysate to be loaded depends on the level of protein expression and may vary between experiments.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of PBS containing 5% non-fat dry milk (PBS, Product No. D 8537; non-fat dry milk, Product No. M 7409) for at least 60 min.
4. Wash the membrane three times for 10 minutes each in PBS containing 0.05% TWEEN® 20 (Product No. P 3563).
5. Incubate the membrane with Anti-MAT antibody as the primary antibody in PBS containing 1% BSA, with agitation for 120 minutes.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20.
7. Incubate the membrane with Anti-Mouse IgG, peroxidase conjugate (Product No. A 9917, A 3682 or A 2304) as the secondary antibody, at the recommended concentration in PBS containing 0.05% TWEEN 20. Incubate for 60 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 10 minutes each in PBS containing 0.05% TWEEN 20.
9. Treat the membrane with a Peroxidase substrate such as the CPS substrate (Product No. PQ0201).

Indirect Immunofluorescent staining of cultured cells

All incubation steps should be performed at room temperature.

1. Grow transfected cultured cells expressing MAT-tagged protein of choice on sterile coverslips at 37 °C.
2. Wash the cells briefly in PBS (Product No. D 8537).
3. Fix with 3% or 4% paraformaldehyde (10 minutes) and permeabilize with 0.5 % Triton® X-100 (2 minutes).
4. Wash coverslips twice in PBS (5 minutes each wash).
5. Incubate coverslips cell-side-up with Anti-MAT in PBS containing 1% BSA (Product No. A 9647) for 60 minutes.
6. Wash three times in PBS (5 minutes each wash).
7. Incubate coverslips cell-side-up with Anti-Mouse FITC conjugate (e.g. Product No. F 4018 or F 8771) as the secondary antibody, at the recommended dilution, in PBS containing 1% BSA, for 30 minutes.
8. Wash three times in PBS (5 minutes each wash).
9. Add one drop of aqueous mounting medium on the cover slip and invert carefully on a glass slide. Avoid air bubbles.
10. Examine using a fluorescence microscope with appropriate filters.

Immunoprecipitation

Immunoprecipitation can be easily performed using the Protein G Immunoprecipitation kit (Product No. IP-50) .

1. Centrifuge 20 µl of a 1:1 suspension of Protein G-agarose beads for 1 minute at 2000 *x g*, and then wash twice with 1 ml of IP buffer 1X.
2. Add anti-MAT antibody diluted in PBS to Protein G-agarose beads. Incubate and mix by swinging head-over-tail for 1 hour at room temperature.
3. Centrifuge for 1 minute at 12,000 *x g*, and wash twice with 1 ml IP buffer 1X at 4 °C by spinning.
4. Add 0.1-1.0 ml of cell extract containing MAT tagged protein to the antibody-coupled beads (see Note), and incubate from 2 hours to overnight at 4 °C, while swinging head-over tail.
   Note: The amount of cell extract depends on the level of expression of the tagged protein and the specific application.

5. Spin down beads; remove supernatant.
6. Wash beads four times with 1ml of IP buffer 1X and once with PBS by vortex and short spin.
7. Resuspend the pellet in 25 µl of 2X SDS-PAGE sample buffer. Boil sample for 5 minutes. Centrifuge and remove supernatant to a clean tube. The sample is ready to be loaded on an SDS-PAGE gel.

References

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