Product Information

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Monoclonal Anti-MAT-Tag™
Clone MAT1-87
produced in mouse, purified immunoglobulin

Catalog Number **M6693**

Monoclonal Anti-MAT-Tag (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-Tag peptide HNHRHKHGGGC conjugated to KLH.

Monoclonal Anti-MAT-Tag recognizes the seven amino acid MAT-Tag 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-Tag fusion proteins using metal-based affinity chromatography such as the highly selective HIS-Select® Nickel Affinity Gel, Catalog Number P6611. The MAT-Tag sequence can be incorporated into both prokaryotic and eukaryotic expression vectors. A variety of expression vectors are available from Sigma-Aldrich, e.g., Catalog Numbers E5530, E5780, E5280, C5864, and T6699.

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

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

Antibody concentration: ~2 mg/ml.

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
For continuous use, store at 2-8 °C for up to one month. For extended storage freeze in working aliquots. Repeated freezing and thawing, or 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
Immunoblotting: a working concentration of 1- 2 µg/ml is determined using cell extracts expressing a C-terminal MAT-tagged protein.

Immunoprecipitation: 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 for at least 60 min. PBS, Catalog Number D8537; non-fat dry milk, Catalog Number M7409.

4. Wash the membrane three times for 10 minutes each in PBS containing 0.05% TWEEN® 20, Catalog Number P3563.
5. Incubate the membrane with Monoclonal Anti-MAT-Tag 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, Catalog Numbers A9917, A3682 or A2304, 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 suitable peroxidase substrate.

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.
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 Monoclonal Anti-MAT-Tag in PBS containing 1% BSA, Catalog Number A9647, for 60 minutes.
6. Wash three times in PBS (5 minutes each wash).
7. Incubate coverslips cell-side-up with Anti-Mouse-FITC, e.g., Catalog Numbers F4018 or F8771, 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, Catalog Number IP50.

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 Monoclonal Anti-MAT-Tag 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, 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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