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Product Information

Monoclonal Anti-T7 tag, Clone T7 Tag
produced in mouse, purified immunoglobulin

Catalog Number **T8823**

Product Description

Monoclonal Anti-T7 tag (mouse IgM isotype) is derived from the T7 Tag hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to the T7 tag (MASMTGGQQMG-K) conjugated to KLH. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2.

Monoclonal Anti-T7 tag recognizes the T7 tag sequence on T7-tagged fusion proteins. The product is useful in ELISA and immunoblotting.

Recombinant DNA technology enables the fusion of genes of interest to specific sequences that provide “affinity handles” (tags), designed to enable the selective identification and purification of the protein of interest.[1-5] The addition of a tag to a given gene creates a stable fusion product that does not appear to interfere with the bioactivity of the protein or with the biodistribution of the tagged product.

The T7 tag is an 11 amino acid peptide encoded in the leader sequence of T7 bacteriophage *gene10.* This gene encodes a T7 major capsid protein whose function is not clear. The T7 tag serves as a tag in many expression vectors including the pET system that is based on the very efficient T7 RNA polymerase expression system.[6] Monoclonal antibodies specific for T7 tag are an important tool for studying expression of recombinant T7-tagged proteins.

Reagent

Supplied as a solution in 0.01 M 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 recombinant T7-tagged β-galactosidase.

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

Procedure for Immunoblotting

All incubation steps should be performed at room temperature.

1. Separate T7-tagged proteins from sample extract using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load adequate bacterial or mammalian lysate expressing the T7-tag fusion protein. The amount of extract to be loaded per slab or lane depends on the level of protein expression and may vary between experiments.
2. Transfer proteins from the gel to nitrocellulose membrane.

3. Block the membrane using a solution of 5 % non-fat dry milk in phosphate buffered saline, Catalog Number P4739; or prepared with Dulbecco's PBS, Catalog Number D8537, and non-fat dry milk, Catalog Number M7409, for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20, Catalog Number P3563.
5. Incubate the membrane with Monoclonal Anti-T7 tag, using an optimized concentration in PBS containing 0.05% TWEEN 20, for 60-120 minutes.
6. Wash the membrane three times for 15 minutes each in PBS containing 0.05% TWEEN 20.
7. Incubate the membrane with Anti-Mouse IgG (Fab specific)-Peroxidase, Catalog Number A2304, or with Anti-Mouse IgG (Fab specific)–Alkaline Phosphatase, Catalog Number A2179, as the secondary antibody using an optimized concentration in PBS containing 0.05% TWEEN 20, for 60-120 minutes.
   Note: Adjust the antibody concentration as necessary to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 15 minutes each in PBS containing 0.05% TWEEN 20.
9. Treat the membrane with a peroxidase or an alkaline-phosphatase substrate.

References

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