Technical Bulletin

Monoclonal Anti-T7 tag antibody produced in mouse

Purified immunoglobulin, buffered aqueous solution, clone T7 Tag

T8823

Product Description

Recombinant DNA technology enables the fusion of genes of interest to specific sequences that provide "affinity handles" (tags), which are 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*, which encodes the T7 major capsid protein.[6] The T7 tag serves as a tag in many expression vectors, such as the pET system (which is based on the T7 RNA polymerase expression system).[7] Monoclonal antibodies specific for the T7 tag are an important tool to study expression of recombinant T7-tagged proteins.

Monoclonal Anti-T7 tag (mouse IgM isotype) is derived from the T7 Tag hybridoma which is produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice that have been immunized with a synthetic peptide corresponding to the T7 tag (MASMTGGQQMG-K) conjugated to KLH. The isotype is determined by a double diffusion immunoassay that uses Mouse Monoclonal Antibody Isotyping Reagents (Cat. No. ISO2).

Monoclonal Anti-T7 tag recognizes the T7 tag sequence on T7-tagged fusion proteins. The product has been tested and reported[8] for use in immunoblotting. It may be also used for immunoprecipitation[9] or for ELISA.[10]

Reagent

This product is 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 (exact value on Certificate of Analysis for particular lot)

Precautions and Disclaimer

Because of the sodium azide content, a Safety Data Sheet for this product has been sent to the attention of the safety officer of your institution. Consult the Safety Data Sheet 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, 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: To obtain best results in different techniques and preparations, we recommend determining optimal working dilution by titration test.

Procedure for Immunoblotting

Note: 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 that expresses 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 (Cat. No. P4739); or alternatively a solution prepared with Dulbecco's PBS (Cat. No. D8537) and non-fat dry milk (Cat. No. M7409), for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20 (Cat. No. 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 (Cat. No. A2304), or with Anti-Mouse IgG (Fab specific)-Alkaline Phosphatase (Cat. No. 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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