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ProductInformation

Monoclonal Anti-T7 Tag
Peroxidase Conjugate
Clone T7tag
Purified Mouse Immunoglobulin

Product Number T 3699

TECHNICAL BULLETIN

Product Description

Anti-T7 Tag, Peroxidase conjugate is a lyophilized preparation of the purified immunoglobulin fraction of monoclonal Anti-T7 Tag (mouse IgM isotype) isolated from ascites fluid of the T7 tag hybridoma, conjugated to horseradish peroxidase (HRP). The antibody is derived from the T7 tag hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a synthetic peptide corresponding to the T7 tag (MASMTGGQQMG-K), conjugated to KLH.

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

Recombinant DNA technology enables the insertion of specific DNA sequences into genes of interest. The inserts provide 'affinity handles' (tags) designed for the selective identification and purification of the protein product of the gene.[1-5] The tag and gene form a stable fusion product that does not appear to interfere with the bioactivity or biodistribution of the protein.

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 the T7 tag are an important tool for studying expression of recombinant T7-tagged proteins.

Reagents

The product is supplied as a lyophilized powder. After reconstitution the solution contains 1% BSA and 0.01% thimerosal in 0.01 M sodium phosphate buffered saline, pH 7.4.

Antibody Concentration: 2.0 to 3.0 mg/ml.
Molar ratio Ab/Enzyme: 0.4 to 0.8
Enzyme activity: at least 50 U/ml.

Precautions and Disclaimer

This product is for research use only. Consult the MSDS for information regarding hazards and safe handling practices.

Preparation Instructions

Reconstitute the vial contents with 0.5 ml of distilled water.

Storage/Stability

Store the lyophilized product at 2 to 8 °C.

For continuous use after reconstitution, keep at 2 to 8 °C for up to 1 month. Working dilutions should be discarded. For extended storage, freeze in working aliquots at –20 °C. Avoid repeated freeze-thaw.

Procedure

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 (PBS, Product No. D 8537; non-fat dry milk, Product No. M 7409) for at least 60 minutes.

4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween 20 (Product No. P 3563).
5. Incubate the membrane with Anti-T7 Tag, Peroxidase conjugate 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. Treat the membrane with a peroxidase substrate.

Product Profile

A minimum working dilution of 1:1000 is determined by immunoblotting of 250 to 500 ng of purified T7-tagged β-galactosidase fusion protein.

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

References

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