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AM26687AF-N Monoclonal Antibody to 6xHistidine Epitope Tag (HHHHHH) - Azide Free

Alternate names: 6xHis-Tag, HHHHHH Tag, HIS6 Tag, His Tag

Quantity: 0.1 mg

Background: A variety of plasmids contain DNA that encodes an N-terminal tag consisting of six histidine (His) residues, followed by an extended multiple cloning sites. The His-Tag fusion protein expression system is commonly used because the 6 x His-Tag on the recombinant proteins allows for efficient coupling to Ni++ affinity resins and purification by a single step chromatography.

Host / Isotype: Mouse / IgG1

Clone: 6C4

Immunogen: Synthetic peptide 6 His

Format: **State:** Liquid Ig fraction
Purification: Protein A agarose
Buffer System: PBS containing 50% glycerol, pH 7.2, without preservatives

Applications: Western blot: 1 µg/ml for chemiluminescence detection system. Details see protocol below.
Not recommended for Immunoprecipitation.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This specific antibody for His-Tag fusion protein is useful for monitoring of the fusion protein expression and affinity purification.

Add. Information: This product was originally produced by MBL International.

Storage: Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings: 1. Porath, J., et al., Protein Express. Purif. 3, 263-281 (1992).

Protocols: **SDS-PAGE & Western Blotting**
1) Mix the sample with equal volume of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4oC.
5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted

with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 6 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

Pictures:

Western blot analysis of His-Azami-Green (1) and His-EGFP (2) using AM26687AF-N.