

Biotinylated T7•Tag[®] Monoclonal Antibody



About the Kits

Biotinylated T7•Tag Monoclonal Antibody

125 µl

69968-3

Description

The T7•Tag Monoclonal Antibody is a mouse monoclonal antibody (IgG_{2b}, κ) directed against the 11 amino acid (MetAlaSerMetThrGlyGlyGlnGlnMetGly) gene 10 leader peptide expressed by many pET vectors, as well as pSCREEN™ and pRSET vectors. The Biotinylated T7•Tag Monoclonal Antibody is covalently labeled with biotin under conditions that optimize the sensitivity of detection on blots. When used with Novagen's Streptavidin AP Conjugate (Cat. No. 69219-3), the biotinylated antibody enables a 5-fold increase in detection sensitivity of target proteins on Western blots compared to an anti-IgG conjugate strategy. The antibody has been qualified for Western blotting and can detect less than 1 ng where the cross-reactivity with bacterial, insect or mammalian cells lysates is negligible. The 125 µl package provides enough antibody for 125 Western blots (10 × 10 cm) and a Positive Control Extract containing a 31.1 kDa T7•Tag fusion protein is provided.

Components

- 125 µl Biotinylated T7•Tag Monoclonal Antibody
- 250 µl T7•Tag Positive Control Extract (containing 31.1 kDa T7•Tag protein)

Storage

Store all components at -20°C.

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1



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Western Blotting

This protocol is for the transfer and detection of proteins on nitrocellulose membranes. Note that PVDF or other hydrophobic blotting membranes may require different blocking conditions (e.g. longer blocking incubations, higher concentration of blocking reagent).

T7•Tag Positive Control Extract

The T7•Tag Positive Control Extract is prepared from an *E. coli* λDE3 lysogen carrying a pSCREEN™-1b(+) recombinant that upon induction produces a 31,102 dalton protein containing the T7•Tag peptide. It is supplied at 2.5 ng protein/μl in 1X sample buffer (75 mM DTT, 62.5 mM Tris-HCl, 7.5% glycerol, 1.5 % SDS, 0.005% bromphenol blue, pH 6.8) and can be loaded directly on a gel after heating. Loading 5 μl on a mini-gel should give a strong band at the indicated size using appropriate detection methods.

Preparation

1. For each standard 10 × 10 cm blot, prepare 20 ml of blocking solution, 3% BSA in 1X TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Tween 20).
2. For each standard 10 × 10 cm blot prepare 160 ml 1X TBST for primary and secondary antibody dilutions and washes.
3. Dilute the Biotinylated T7•Tag Monoclonal Antibody 1:10,000 in 1X TBST, e.g. add 1 μl antibody to 10 ml TBST.
4. Dilute the Streptavidin AP Conjugate (Cat. No. 69219-3) 1:5,000 in 1X TBST, e.g. add 2 μl of antibody into 10 ml TBST.

Protocol

1. The following steps should be performed at room temperature with gentle rocking or agitation during incubations. Use a clean tray and place the membrane protein-side up.
2. Run an SDS-PAGE gel of the T7•Tag fusion protein sample. If using the T7•Tag Positive Control Extract, heat the entire tube for 2 min at 37°C to solubilize the SDS. Transfer 5 μl of the extract to a tube and heat for 3 min at 70°C along with the target fusion protein samples. Spin to collect the sample at the bottom of the tube and load onto a gel. A strong sharp 31.1 kDa band will be detected in the Positive Control Extract sample. Load protein markers in an adjacent lane. Perfect Protein™ (Cat. No. 69959-3) or Trail Mix™ (Cat. No. 70982-3) Western Markers are available from Novagen and require the S-protein AP Conjugate (Cat. No. 69598-3) for detection.
3. Transfer the proteins to nitrocellulose electrophoretically. Any standard device can be used according to the manufacturer's instructions. A standard transfer buffer is 25 mM Tris base, 192 mM glycine, pH 8.3, 20% methanol
4. Remove the membrane from the blotting apparatus and incubate in 20 ml blocking solution at room temperature for 30 min.
5. Incubate the membrane for 30 min at room temperature with 10 ml Biotinylated T7•Tag antibody diluted 1:10,000.
6. Wash the membrane three times for 5 min using 20 ml 1X TBST with gentle shaking at room temperature.
7. Incubate the membrane for 30 min at room temperature with 10 ml Streptavidin AP Conjugate diluted 1:5,000.
8. Wash the membrane three times for 5 min using 20 ml 1X TBST with gentle shaking at room temperature.

Note: Washing steps should be performed in sufficient volume and repeated three times to assure complete removal of unbound conjugate. If background is evident, the blot can be washed several more times before adding additional substrate.

9. Develop the blot with chemiluminescent or colorimetric detection reagents (see below).



Chemiluminescent detection

CDP-Star[®] AP Substrate (Cat. No. 69086-3) is available from Novagen for sensitive chemiluminescent detection. Use 1.5 ml CDP-Star AP Substrate per 10 × 10 cm blot.

1. After the final washing step is complete, try to drain as much TBST from the membrane as possible. It is helpful to touch the corner of a dry paper towel to the edge of the membrane as it is held at an angle. Place the membrane protein-side up in a clean tray or on plastic wrap.
2. For a typical 10 × 10 cm blot, 1.5 ml CDP-Star Substrate is sufficient. Wet the entire surface of the membrane with the appropriate substrate. Incubate the blot in the substrate at room temperature for 1 min.
3. Remove the membrane from the substrate. Drain any excess substrate from the membrane by touching the edge to a paper towel. Place the membrane in a Development Folder (Cat. No. 69137-3) or on a fresh sheet of plastic wrap and fold the plastic over the membrane. Remove any bubbles between the plastic and the membrane. Gently remove any liquid from the exterior of the plastic.
Optional: Place a gLOCATOR[™] Luminescent Label (Cat. No. 69102-3) on a corner of the Development Folder. The gLOCATOR Luminescent Label has space to record blot-identifying data for future reference.
4. Place the wrapped membrane in a film cassette with autoradiographic film and expose for 1–10 min. An initial exposure time of 1 min is recommended. Longer exposures can be performed although the highest light output occurs in the first five minutes. Light output continues over several hours. Be careful not to move the film or membrane after initial placement or multiple images can result.

Colorimetric detection

AP Detection Reagent Kit (Cat. No. 69264-3) is available from Novagen for colorimetric detection and contains enough NBT, BCIP and 20X AP Buffer for 25 blots (10 × 10 cm).

1. Based on a 10 × 10 cm blot, prepare developing solution by combining 60 µl NBT (83 mg/ml nitro-blue tetrazolium in 70% (v/v) dimethylformamide) and 60 µl BCIP (42 mg/ml 5-bromo-4-chloro-3-indoyl phosphate, toluidinium salt in 100% dimethylformamide) to 15 ml of 1X AP buffer (100 mM Tris, pH 9.5, 100 mM NaCl, 1 mM MgCl₂).
2. Place the membrane protein side up in a clean tray and add the developing solution. Incubate the membrane at room temperature until purple color develops. Strong purple signal should appear within 2–10 minutes.
3. To stop the reaction, wash the blot thoroughly in deionized water and allow to air dry. Store dry blot at room temperature wrapped in plastic.

Dot Blot Protocol

1. Make serial dilutions of a prepared extract in 10mM Tris-HCl, 25 mM EDTA, pH 8.0 covering a range of 2 µg/ml – 200 µg/ml protein (make one additional series for the T7•Tag positive Control extract if desired).
2. Spot 1 µl of a T7•Tag fusion protein dilution directly onto nitrocellulose. Allow to air dry for several minutes.

Note: The bromphenol blue dye in the sample buffer of the T7•Tag Positive Control Extract does not interfere with detection. It is washed away prior to the addition of the colorimetric or chemiluminescent reagents.

3. Proceed as described for steps 4–9 under the Western Blot Protocol.