S•Tag™ Monoclonal Antibody

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USA and Canada
Tel (800) 628-8470
biosciencehelp@
emdchemicals.com

Europe

France
Freephone
0800 126 461

Germany
Freecall
0800 100 3496

Ireland
Toll Free
1800 409 445

United Kingdom
Freephone
0800 622 935

All other European Countries
+44 115 943 0840

techservice@merckbio.eu

All Other Countries
Contact Your Local Distributor
www.merck4biosciences.com
biosciencehelp@
emdchemicals.com

www.merck4biosciences.com

About the Kits

S•Tag™ Monoclonal Antibody 71549-3

Description

The S•Tag™ Monoclonal Antibody is a mouse monoclonal antibody (IgG_{2b}) directed against the 15 aa S•Tag peptide encoded by many Novagen® vectors. This Protein G purified antibody is suitable for detecting fusion proteins that contain an S•Tag by immunoblotting and can also be used for immunoprecipitation and immunofluorescence.

The S•Tag Monoclonal Antibody can detect as little as 1 ng of S•Tag fusion proteins with negligible cross-reactivity with bacterial, insect, or mammalian lysates.

The 50 µg package provides enough antibody to perform 50 Western blots (10 x 10 cm) or 20 immunoprecipitation assays.

Components

- 50 µg S•Tag™ Monoclonal Antibody (1 mg/ml in PBS, 50% glycerol, 0.02% sodium azide)

Storage

Store at –20°C.

Protocols

Western Blotting

This protocol is for the detection of proteins on nitrocellulose membranes. 5% nonfat milk in 1X TBST (150 mM NaCl, 10 mM Tris-HCl, 0.1% TWEEN®-20, pH 7.5) is recommended for blocking. This combination of buffer and blocking reagent results in the lowest background and can be used throughout the protocol. Note, PVDF or other hydrophobic blotting membranes may require different blocking conditions (e.g., increased incubation time, increased blocking reagent concentration). This protocol is based on a 10 × 10 cm blot. Larger or smaller membranes require adjustment of reagent volumes.

Preparation

1. Prepare 250 ml 1X TBST per blot. Combine 25 ml 10X TBST and deionized water for a final volume of 250 ml.
2. Prepare 30 ml fresh blocking solution per blot for washing the membrane. Combine 1.5 g nonfat dried milk with 3 ml 10X TBST and add deionized water to a final volume of 30 ml.
3. Dilute 2 µl S•Tag™ Monoclonal Antibody 1:5000 (v/v) in 1X TBST for a final volume of 10 ml.
4. Dilute Goat Anti-Mouse IgG AP or HRP conjugate in 1X TBST according to the manufacturer's instructions for a final volume of 10 ml (e.g., 1 µl conjugate into 10 ml 1X TBST, 1:10,000 v/v). For added convenience, the Novagen® Goat Anti-Mouse IgG AP Conjugate (Cat. No. 69266) and Goat Anti-Mouse IgG HRP Conjugate (Cat. No. 71045) are separately available.

Protocol

The following steps should be performed at room temperature, with gentle rocking or agitation during incubations. Use a clean tray and place membrane protein-side up.

1. Run SDS-polyacrylamide gel of S•Tag fusion protein sample. Load protein size markers in an adjacent lane. Perfect Protein™ Western Markers (Cat. No. 69959) or Trail Mix™ Western Markers (Cat. No. 70982) are available and require an S-protein AP Conjugate (Cat. No. 69598) or S-protein HRP Conjugate (Cat. No. 69047) for detection. If using Trail Mix Western Markers, combine 5 µl Trail Mix Western Markers and 1 µl ß-mercaptoethanol. This lane will display bands at 15, 25, 35, 50, 75, 100, 150, and 225 kDa when detected with kit reagents. Trail Mix Western Markers also include three prestained proteins at 100, 16, and 15 kDa to allow direct visualization of protein mobility during electrophoresis.

   Note: Do not heat Perfect Protein Western Markers prior to loading.

2. Electrophoretically transfer proteins to nitrocellulose. Any standard device can be used according to the manufacturer instructions. Due to the large size of 150 kDa and 225 kDa bands, incomplete transfer may be observed. The 15 kDa band may not efficiently bind to the membrane (particularly 0.45 µm pore size nitrocellulose) due to its small size.

   Note: The standard transfer buffer is 192 mM glycine, 25 mM Tris base, 20% methanol, pH 8.0.

3. Incubate for 30 min in 30 ml blocking solution.
4. Wash three times, each for 5 min, with 20 ml 1X TBST.
5. Incubate for 30 min in 10 ml S•Tag Monoclonal Antibody diluted 1:5000 in 1X TBST.
6. Wash three times, each for 5 min, with 20 ml 1X TBST.
7. Incubate 30 min with 10 ml Goat Anti-Mouse IgG Conjugate diluted 1:10,000 in 1X TBST.
8. Wash five times, each for 5 min, with 20 ml 1X TBST.

   Note: Thoroughly wash membrane to achieve maximum signal:noise ratios. Washing steps should be performed in sufficient volume and repeated five 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.

Chemiluminescent Detection

1. Drain as much TBST from membrane as possible. It is helpful to touch the corner of a dry paper towel to edge of membrane as it is held at an angle. Place damp membrane, protein side up, on sheet of plastic wrap, or in clean tray.
2. Prepare substrate immediately before use. For a typical 10 × 10 cm blot, 1.5 ml CDP-*Star*® AP Substrate, or 1 ml SuperSignal® HRP Substrate working solution is a sufficient volume. Prepare SuperSignal Substrate by combining equal parts 2X Luminol/Enhancer and 2X Stable Peroxide Solution and mixing briefly. Wet entire surface of membrane with appropriate substrate. Incubate blot in substrate at room temperature for 1 min.
3. Remove membrane from substrate. Drain excess substrate from membrane by touching membrane edge to a paper towel. Place membrane in Development Folder or on a fresh sheet of plastic wrap, and fold the plastic over membrane. Remove any bubbles between plastic and membrane. Gently remove any liquid from exterior of the plastic.
4. **Optional**: Place a gLOCATOR™ Luminescent Label (Cat. No. 69102) on a corner of Development Folder and record blot-identifying data for future reference.
5. Place wrapped membrane in film cassette with autoradiographic film. Expose for 1–10 min. Do not move film or membrane after initial placement or multiple images can result. We recommend an initial exposure time of 1 min. Light output continues for several hours. Extended exposures can be performed, although the highest light output occurs in the first 5 min.

Colorimetric detection for Alkaline Phosphatase (AP)

1. Prepare developing solution by adding 60 µl NBT solution and 60 µl BCIP solution to 15 ml 1X AP buffer. AP Detection Kit (Cat. No. 69264), which contains these components, is separately available.
2. Place membrane in clean tray and add developing solution. Incubate membrane at room temperature until color develops. Strong purple signals should appear within 2–10 min.
3. To stop reaction, wash blot thoroughly in deionized water. Allow to air dry. Store dry blots at room temperature wrapped in plastic.

Dot Blotting

1. Make serial dilutions of a prepared extract in sterile, deionized water. The dilutions should cover a range of 1–80 ug/ml protein.
2. Spot 1 µl of each diluted S•Tag™ fusion protein sample directly onto nitrocellulose. Allow to air dry for several minutes.
3. Proceed as described for steps 3–9 of the Western blotting protocol (see p 3).

Immunoprecipitation

The following protocols are for use with the GrabIt™ Protein G Plus/Protein A Kit (Cat. No. 71436).

Preabsorption procedure (Preabsorption is often performed to minimize extra bands resulting from nonspecific binding.)

1. Place 1 ml fusion protein sample in 1.5 ml microcentrifuge tube.
2. **Optional:** Add 2.5 µg normal mouse IgG to the sample, and mix by inverting tube. Place tube on rocker or spin wheel at low rpm at 4°C for 30 min.
3. Add 50 µl Protein G Plus/Protein A Agarose slurry to extract and mix by inverting tube. Place tube on rocker or spin wheel at low rpm at 4°C for 1 h.
4. Place Spin Filter in 2 ml Receiver Tube. Transfer 500 µl extract/resin mixture to Spin Filter.
5. Spin Receiver Tube/Spin Filter at 14,000 x g for 30 s.
6. Transfer flow-through lysate from receiver tube to 1.5 ml microcentrifuge tube.
7. Add remaining extract/agarose mixture to Spin Filter and spin, as in Step 5.
8. Pool preabsorbed lysates.

Immunoprecipitation

1. Place 1 ml sample, or 1 ml preabsorbed lysate in a 1.5 ml microcentrifuge tube.
2. Add 2.5 µg S•Tag™ Monoclonal Antibody to the extract, and mix by inverting tube. Place tube on rocker or spin wheel at low rpm at 4°C for 30 min.
3. Add 50 µl Protein G Plus/Protein A Agarose slurry to extract, and mix by inverting tube. Place tube on rocker or spin wheel at low rpm at 4°C for 1 h.
4. Prepare 100 µl 1X SDS sample buffer (75 mM DTT, 62.5 mM Tris-HCl, 7.5% glycerol, 1.5% SDS, 0.005% bromophenol blue, pH 6.8) per reaction by combining 25 µl 4X SDS Sample Buffer (Cat. No. 70607) and 75 µl water. Heat to 80°C.
5. Place Spin Filter in 2 ml Receiver Tube. Transfer 500 µl extract/resin mixture to Spin Filter.
6. Spin Receiver Tube/Spin Filter at 14,000 x g for 30 s.

   Note: Do not let resin dry during procedure. Drying can reduce yield of target proteins. After centrifugation, proceed immediately to the next step.
7. Discard flow-through.
8. Add remaining extract/agarose mixture to Spin Filter and spin, as in Step 5.
9. Empty Receiver Tube. Wash resin three times by adding 500 µl GrabIt™ Wash Buffer directly to agarose. Between washes, spin Receiver Tube/Spin Filter at 14,000 x g for 30 s. Empty Receiver Tube after each spin.
10. Transfer Spin Filter to new Receiver Tube.
11. Add 50 µl preheated 1X SDS sample buffer (prepared in Step 4) to agarose. Cap Receiver Tube. Place at 80°C for 5 min.
12. Spin Receiver Tube/Spin Filter at 14,000 x g for 30 s.
13. Sample can be immediately loaded on gel for SDS-PAGE analysis, or stored at –20°C.