

# His•Tag<sup>®</sup> Monoclonal Antibody

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## About the Kits

|                               |          |         |
|-------------------------------|----------|---------|
| His•Tag® Monoclonal Antibody  | 100 µg   | 70796-3 |
|                               | 3 µg     | 70796-4 |
| His•Tag AP Western Reagents   | 25 blots | 70972-3 |
| His•Tag AP LumiBlot™ Reagents | 25 blots | 70973-3 |
| His•Tag HRP LumiBlot Reagents | 25 blots | 70974-3 |

### Description

The His•Tag Monoclonal Antibody is a mouse monoclonal antibody (IgG<sub>1</sub>) directed against the His•Tag sequence encoded by many of the Novagen expression vectors, as well as many other commercially available vectors. The His•Tag Monoclonal Antibody recognizes five consecutive histidine residues (HHHHH) regardless of the surrounding amino acid context. The high affinity ( $K_d = 5 \times 10^{-8}$ – $1 \times 10^{-9}$  M) enables sensitive, specific detection of His•Tag fusion proteins at antibody concentrations of 0.1–0.2 µg/ml. The His•Tag Monoclonal Antibody binds to N-terminal, C-terminal, and internal His•Tag sequences. The His•Tag Monoclonal Antibody also detects the recombinant marker bands in the Novagen Perfect Protein™ or Trail Mix™ Western Markers for convenient visualization of accurate internal standards on Western blots.

Using standard colorimetric detection methods with His•Tag AP Western Reagents, as little as 2 ng protein can be detected on Western blots, or 0.5 ng protein on dot blots. Chemiluminescent detection methods with His•Tag AP or HRP LumiBlot Reagents may be up to 10-fold more sensitive. Cross-reactivity to bacterial, yeast, insect or mammalian cell proteins is negligible.

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## Components

### His•Tag® Monoclonal Antibody

- 100 or 3 µg His•Tag Monoclonal Antibody (lyophilized)

### His•Tag AP Western Reagents

- 125 ml 20X TBS (3 M NaCl, 200 mM Tris-HCl, pH 7.5)
- 2 × 250 ml 10X TBSTT (5 M NaCl, 200 mM Tris-HCl, 2% v/v Triton® X-100, 0.5% v/v Tween®-20, pH 7.5)
- 25 g Albumin, Bovine Serum
- 40 µl Goat Anti-Mouse IgG AP Conjugate (H + L)
- 1.5 ml BCIP at 42 mg/ml in 100% dimethylformamide
- 1.5 ml NBT at 83 mg/ml in 70% dimethylformamide
- 20 ml 20X AP Buffer
- 125 µl Trail Mix™ Western Markers

### His•Tag AP LumiBlot™ Reagents

- 125 ml 20X TBS (3 M NaCl, 200 mM Tris-HCl, pH 7.5)
- 2 × 250 ml 10X TBSTT (5 M NaCl, 200 mM Tris-HCl, 2% v/v Triton X-100, 0.5% v/v Tween-20, pH 7.5)
- 225 ml 5% Alkali-Soluble Casein
- 40 µl Goat Anti-Mouse IgG AP Conjugate (H + L)
- 40 ml CDP-Star® AP Substrate with Nitro-Block™ II
- 25 gLOCATOR™ Luminescent Labels
- 25 Development Folders
- 125 µl Trail Mix Western Markers

### His•Tag HRP LumiBlot Reagents

- 125 ml 20X TBS (3 M NaCl, 200 mM Tris-HCl, pH 7.5)
- 2 × 250 ml 10X TBSTT (5 M NaCl, 200 mM Tris-HCl, 2% v/v Triton X-100, 0.5% v/v Tween-20, pH 7.5)
- 225 ml 5% Alkali-Soluble Casein
- 40 µl Goat Anti-Mouse IgG HRP Conjugate (H + L)
- 25 ml 2X Stable Peroxide Solution
- 25 ml 2X Luminol/Enhancer Solution
- 25 gLOCATOR Luminescent Labels
- 25 Development Folders
- 125 µl Trail Mix Western Markers

## Storage

His•Tag Monoclonal Antibody is supplied as lyophilized powder, and is stable for one year when stored at 4°C. Resuspended antibody may be stored at 4°C for up to three months, or frozen in aliquots at -20°C for up to six months. Avoid repeated freezing and thawing.

Store Goat Anti-Mouse IgG AP or HRP Conjugate (H+L), BCIP, and NBT at -20°C. Store Development Folders and gLOCATOR Luminescent Labels at room temperature. Store 20X TBS, 10X TBSTT, 5% Alkali-Soluble Casein, Bovine Serum Albumin (BSA), 20X AP Buffer, CDP-Star AP Substrate, 2X Stable Peroxide Solution and 2X Luminol/Enhancer Solution at 4°C.

Store Trail Mix and Perfect Protein Western Markers at -20°C or 4°C. For long term storage (> 6 months), we recommend the markers be stored at -20°C. If a precipitate forms during storage at -20°C, heat at 37°C for 10 min. Avoid repeatedly heating the markers to 37°C, which may compromise product quality. For frequent use, store the markers at 4°C or at -20°C in working aliquots.

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## Resuspension of His•Tag<sup>®</sup> Monoclonal Antibody

The His•Tag Monoclonal Antibody is provided as a lyophilized powder and must be resuspended prior to use in the following protocols. Dissolve the lyophilized antibody in 500 µl sterile water per 100-µg vial or 15 µl sterile water per 3-µg vial (final concentration 0.2 mg/ml).

## Chemiluminescent Detection

We recommend Alkali-Soluble Casein as the blocking reagent for chemiluminescent detection on nitrocellulose membranes because it results in the lowest background and can be used as a blocking reagent throughout the protocol.

*Note: PVDF or other hydrophobic blotting membranes may require different blocking conditions (e.g., increased incubation time, increased blocking reagent concentration).*

### Preparation

#### Alkali-Soluble Casein

Alkali-Soluble Casein is available as a 5% solution (Cat. No. 70955-3), or as a powder (Cat. No. 210680). Refer to Appendix (page 7) for preparation of 5% Alkali-Soluble Casein from powder.

1. For each standard 10 × 10 cm blot, prepare 45 ml blocking solution (1% Alkali-Soluble Casein in 1X TBS) per blot by mixing 9 ml 5% Alkali-Soluble Casein and 36 ml deionized water. Fresh blocking solution should be prepared each time. This blocking solution will also be used for primary and secondary antibody dilution.
2. For each standard 10 × 10 cm blot, prepare 60 ml 1X TBS. Combine 3 ml 20X TBS and deionized water for a final volume of 60 ml.
3. For each standard 10 × 10 cm blot, prepare 180 ml 1X TBSTT. Combine 18 ml 10X TBSTT and deionized water for a final volume of 180 ml.
4. Dilute resuspended His•Tag Monoclonal Antibody 1:1000 (v/v) in blocking solution for a final volume of 10 ml.
5. Dilute the Goat Anti-Mouse IgG AP or HRP conjugate 1:5000 (v/v) in blocking solution for a final volume of 8 ml.

### Western blot protocol

The following steps should be performed at room temperature (20–21°C), with gentle rocking or agitation during incubations. Use a clean tray and place membrane protein-side up. This protocol is based on a 10 × 10 cm blot; larger or smaller membranes will require adjustment of reagent volumes.

1. If using Trail Mix™ Western Markers, combine 5 µl Trail Mix Western Markers and 1 µl β-mercaptoethanol.
2. Run SDS-PAGE of His•Tag fusion protein sample. Load Trail Mix Western Markers plus β-mercaptoethanol from Step 1, or load 5 µl Perfect Protein™ Western Markers. 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.*

*Note: We do not recommend detection of Trail Mix Western Markers with His•Tag Monoclonal Antibody and Goat Anti-Mouse IgG HRP Conjugate (H + L). As an alternative, we recommend Perfect Protein Western Markers.*

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3. Electrophoretically transfer proteins to nitrocellulose. Any standard device can be used according to the manufacturer instructions. Due to the large size of the 150- 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.

4. Wash membrane two times, each for 10 min, with 15 ml 1X TBS.
5. Incubate for 1 h in 27 ml blocking solution.
6. Wash two times, each for 10 min, with 20 ml 1X TBSTT.
7. Wash for 10 min with 15 ml 1X TBS.
8. Incubate for 1 h in 10 ml His•Tag® Monoclonal Antibody diluted 1:1000 in blocking solution.
9. Wash two times, each for 10 min, with 20 ml 1X TBSTT.
10. Wash for 10 min with 15 ml 1X TBS.
11. Incubate for 1 h with 8 ml Goat anti-Mouse IgG AP or HRP Conjugate diluted 1:5000 in blocking solution.
12. Wash five times, each for 10 min, with 20 ml 1X TBSTT.

*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.

13. Drain as much TBSTT 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.
14. 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.
15. 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. Place a gLOCATOR™ Luminescent Label on a corner of Development Folder and record blot-identifying data for future reference.
16. 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.

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## Colorimetric detection

Bovine Serum Albumin (BSA) is the recommended blocking reagent for colorimetric detection on nitrocellulose membranes. In some cases, 1% Alkali-Soluble Casein may be used to reduce non-specific background.

### Preparation

1. For each standard 10 × 10 cm blot, prepare 33.3 ml blocking solution (3% BSA in 1X TBS) by mixing 1 g BSA, 1.65 ml 20X TBS and 90% final volume deionized water. After BSA is dissolved, bring to volume with deionized water. Fresh blocking solution should be prepared each time. This blocking solution will also be used for the primary and secondary antibody dilution.
2. For each standard 10 × 10 cm blot, prepare 60 ml 1X TBS. Combine 3 ml 20X TBS and deionized water for a final volume of 60 ml.
3. For each standard 10 × 10 cm blot, prepare 180 ml of 1X TBSTT. Combine 18 ml of TBSTT and deionized water for a final volume of 180 ml.
4. Dilute resuspended His•Tag<sup>®</sup> Monoclonal Antibody 1:1000 (v/v) in blocking solution for a total of 10 ml.
5. Dilute Goat Anti-Mouse IgG AP Conjugate 1:5000 (v/v) in blocking solution for a total of 8 ml.

### Western blot protocol

The following steps should be performed at room temperature (20–21°C), with gentle rocking or agitation during incubations. Use a clean tray and place the membrane protein-side up. This protocol is based on a 10 × 10 cm blot, larger or smaller membranes will require adjusting the volumes.

1. If using Trail Mix<sup>™</sup> Western Markers, combine 5 µl Trail Mix Western Markers and 1 µl β-mercaptoethanol.
2. Run SDS-PAGE of the His•Tag fusion protein sample. Load Trail Mix Western Markers plus β-mercaptoethanol from Step 1, or load 5 µl Perfect Protein<sup>™</sup> Western Markers. This lane will display bands at 15, 25, 35, 50, 75, 100, 150, and 225 kDa when detected with the 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.

*Note:* We do not recommend detection of Trail Mix Western Markers with His•Tag Monoclonal Antibody and Goat Anti-Mouse IgG HRP Conjugate (H + L). As an alternative, we recommend Perfect Protein Western Markers.

3. Electrophoretically transfer proteins to nitrocellulose. Any standard device can be used according to the manufacturer instructions. Due to the large size of the 150- and 225-kDa band, incomplete transfer may be observed. The 15-kDa band may not efficiently bind to the membrane (particularly 0.45 µ 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.

4. Wash membrane two times, each for 10 min, with 15 ml 1X TBS.
5. Incubate for 1 h in 15 ml blocking solution.
6. Wash two times, each for 10 min, with 20 ml 1X TBSTT.
7. Wash for 10 min with 15 ml 1X TBS.
8. Incubate for 1 h in 10 ml His•Tag<sup>®</sup> Monoclonal Antibody diluted 1:1000 in blocking solution.
9. Wash two times, each for 10 min, with 20 ml 1X TBSTT.
10. Wash for 10 min with 15 ml 1X TBS.

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11. Incubate for 1 h with 8 ml Goat Anti-Mouse IgG AP Conjugate diluted 1:5000 in blocking solution.
12. Wash five times, each for 10 min, 20 ml 1X TBSTT.

*Note:* Thoroughly wash membrane to achieve maximum signal:noise ratios.

13. Prepare developing solution by adding 60 µl NBT solution and 60 µl BCIP solution to 15 ml 1X AP buffer.
14. 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.
15. To stop reaction, wash blot thoroughly in deionized water. Allow to air dry. Store dry blots at room temperature wrapped in plastic.

## Appendix

Prepare 5% Alkali-Soluble Casein in 5 X TBS from dry powder (Calbiochem Cat. No. 218680) as follows:

1. Prepare 1 M Tris-HCl, pH 6.0 and 5 M NaCl solutions.
2. Add solid casein to 70% of final volume of deionized water and mix well. Final concentration will be 5g/100 ml. Allow casein to hydrate for at least 10 min. At this point, casein is not solubilized. A homogenous suspension indicates complete hydration.
3. Add 10 M NaOH in small increments to solubilize casein. As NaOH is added, allow solution to equilibrate thoroughly. This process may take several hours. Avoid adding excess NaOH or achieving the correct pH will be difficult. Approximately 350 µl is needed per 100 ml.
4. When casein is completely in solution, add appropriate amounts of 1 M Tris (5ml/100ml; final concentration 50 mM) and NaCl (15ml/100ml; final concentration 750 mM).
5. Adjust the pH to 7.5 with NaOH. Bring to final volume with deionized water.
6. Store at 4°C.

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