Nus•Tag™ Monoclonal Antibody

About the Kit

Nus•Tag Monoclonal Antibody	50 µg	71127-3
	250 µg	71127-4

Description

The Nus•Tag Monoclonal Antibody is a mouse monoclonal antibody (IgG_1) with high affinity for the 54.8 kDa NusA protein (Nus•Tag) from *E. coli* (1, 2). This purified antibody is specifically detects fusion proteins containing the Nus•Tag sequence. The 50 µg package size provides enough antibody for 50 Western blots (10 cm × 10 cm).

Specificity	495 aa NusA protein, precise epitope not determined
Species/isotype	Mouse monoclonal IgG_1
Cross-reactivity	Negligible with bacterial, insect or mammalian cell lysates
Sensitivity	2–5 ng: Western blot developed with chromogenic substrates
	< 1 ng: Western blot developed with chemiluminescent substrate
Form	Stabilized solution of antibody (1 mg/ml) in 50% glycerol
Working dilution	1:10,000 for Western blotting

Components

- 50 µg or 250 µg Nus•Tag Monoclonal Antibody

Storage

Store the Nus•Tag Monoclonal Antibody at –20°C.

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Chemiluminescent detection

This protocol is for the detection of proteins on nitrocellulose membranes. Alkali-Soluble Casein is the recommended blocking reagent because it results in the lowest background. Gelatin can be used for greater sensitivity but may result in a higher background.

Preparation

1. Prepare fresh blocking solution for washing the membrane. For each standard 10 × 10 cm blot, prepare 20 ml of blocking solution. Either dilute 5% Alkali-Soluble Casein prepared with 5X TBS (Cat. No. 70955-3) to 1% with deionized water or prepare a stock of Alkali-Soluble Casein (Calbiochem Cat. No. 218680) according to Appendix A, page 4. Alternatively, prepare 1% Gelatin in 1X TBST as the blocking solution.
2. Prepare 40 ml fresh blocking solution for the primary and secondary antibody dilutions per standard 10 × 10 cm blot. Novagen recommends 0.5% Alkali-Soluble Casein in 0.5X TBS. Dilute 5% Alkali-Soluble Casein prepared with 5X TBS (Cat. No. 70955-3) to 0.5% using deionized water. Alternatively, prepare 0.5% Gelatin in 1X TBST as the blocking solution.
3. Prepare 40 ml 1X TBS (150 mM NaCl, 10 mM Tris-HCl, pH 7.5) and 180 ml X TBSTT (500 mM NaCl, 20 mM Tris-HCl, 0.2% v/v Triton® X-100, 0.05% v/v Tween®-20, pH 7.5) per standard 10 × 10 cm blot.
4. Dilute the Nus•Tag Monoclonal Antibody 1:10,000. Dilute 2 µl of antibody into 20 ml of blocking solution.
5. Dilute the Goat Anti-Mouse IgG AP (Cat. No. 69266-3) or HRP (Cat. No. 71045-3) Conjugate 1:5,000 in blocking solution. Dilute 4 µl of antibody into 20 ml of blocking solution.

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 a SDS-polyacrylamide gel of the Nus•Tag fusion protein sample. Load protein size 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 an S-protein AP or HRP Conjugates (AP; Cat. No.69598-3, HRP; Cat. No. 69047-3) or the His•Tag® Monoclonal Antibody (Cat. No. 70796-3) for detection.

Note: *Detection of Trail Mix Western Markers with the His•Tag Monoclonal Antibody and Goat Anti-Mouse IgG HRP Conjugate (H+L) is not recommended. As an alternative, use Perfect Protein Western Markers with this conjugate.*

3. Transfer the proteins to a membrane electrophoretically. Any standard device can be used according to the manufacturer's instructions. The standard transfer buffer is 25 mM Tris Base, 192 mM glycine, pH 8.3, 20% methanol. If using the Perfect Protein or Trail Mix Western markers, the 150 and 225 kDa bands may transfer incompletely due to their large size. The 15 kDa band may not efficiently bind to the membrane (particularly 0.45 µm pore size nitrocellulose) due to its small size.
4. Incubate for 60 min in 20 ml blocking solution.

Note: *PVDF or other hydrophobic membranes may require different blocking conditions (e.g. longer blocking times, higher concentrations of blocking reagent).*

5. Wash twice for 5 min each time with 20 ml 1X TBSTT.
6. Wash for 5 min with 20 ml 1X TBS.
7. Incubate for 1 h with 20 ml Nus•Tag Monoclonal Antibody diluted 1:10,000 in 0.5% Alkali-Soluble Casein blocking solution.
8. Wash twice for 5 min each time with 20 ml 1X TBSTT.
9. Wash for 5 min with 20 ml 1X TBS.
10. Incubate for 1 h with 20 ml Goat Anti-Mouse IgG AP or HRP Conjugate in 0.5 % Alkali-Soluble Casein blocking solution.
11. Wash five times for 5 min each with 20 ml 1X TBSTT. It is important to thoroughly wash the membrane at this point to achieve maximum signal:noise ratios.

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Note: *Washing steps should be performed in sufficient volume and repeated 5 times to assure complete removal of unbound conjugate. If background is evident, the blot can be washed several more times before adding additional substrate.*

12. After the final washing step is complete, try to drain as much TBSTT 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. Use plastic wrap or a clean tray and place the membrane protein side up.
13. For a typical 10 × 10 cm blot, 1–1.5 ml of the chemiluminescent substrate working solution is sufficient. Prepare the substrate immediately before use. Wet the entire surface of the membrane with the appropriate substrate. Incubate the blot in the substrate at room temperature for 1 minute.

Note: *CDP-Star® AP Substrate (69086-3) or SuperSignal® HRP Substrate (69059-3) are available from Novagen for sensitive chemiluminescent detection. Use 1.5 ml of the CDP-Star® AP Substrate or 1 ml of the SuperSignal® HRP Substrate. Prepare the SuperSignal Substrate working solution by combining equal parts 2X Luminol/Enhancer and 2X Stable Peroxide Solution and mixing briefly.*

14. 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.
15. 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.

Note: *Bacterial extracts contain endogenous 54.8 kDa NusA protein that may be detected.*

Colorimetric detection

This protocol is for the detection of proteins on nitrocellulose membranes. Bovine Serum Albumin (BSA) or gelatin are the recommended blocking reagents. In some cases, 1% Alkali-Soluble Casein may be used to reduce non-specific background. Note that using Alkali-Soluble Casein may reduce sensitivity.

Preparation

1. Prepare 20 ml fresh blocking solution for washing the membrane per standard 10 × 10 cm blot. Use 3% BSA or 1% Gelatin in 1X TBST (150 mM NaCl, 10 mM Tris-HCl, 0.1% v/v Tween®-20, pH 7.5).
2. Prepare 40 ml fresh blocking solution for diluting the primary and secondary antibodies. Use 1.5% BSA or 0.5% Gelatin in 1X TBST.
3. Prepare 40 ml 1X TBS (150 mM NaCl, 10 mM Tris-HCl, pH 7.5) and 180 ml 1X TBSTT (500 mM NaCl, 20 mM Tris-HCl, 0.2% v/v Triton® X-100, 0.05% v/v Tween®-20, pH 7.5) per standard 10 × 10 cm blot.
4. Dilute the Nus•Tag Monoclonal Antibody 1:10,000. Dilute 2 µl of antibody into 20 ml of blocking solution.
5. Dilute the Goat Anti-Mouse IgG AP (Cat. No. 69266-3) or HRP (Cat. No. 71045-3) Conjugate 1:5,000 in blocking solution. Dilute 4 µl of antibody into 20 ml of blocking solution.

Protocol

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.

1. Run a SDS-polyacrylamide gel of the Nus•Tag fusion protein sample. Load protein size 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 an S-protein AP or HRP Conjugates (AP; Cat. No.69598-3, HRP; Cat. No. 69047-3) or the His•Tag® Monoclonal Antibody (Cat. No. 70796-3) for detection.
2. Transfer the proteins to a membrane electrophoretically. Any standard device can be used according to the manufacturer's instructions. The standard transfer buffer is 25 mM Tris Base, 192 mM glycine, pH 8.3, 20% methanol. If using the Perfect Protein or Trail Mix Western markers, the 150 and 225 kDa bands may transfer incompletely due to their large size. The 15 kDa band may not efficiently bind to the membrane (particularly 0.45 µ pore size nitrocellulose) due to its small size.

Note: *Detection of Trail Mix Western Markers with the His•Tag Monoclonal Antibody and Goat Anti-Mouse IgG HRP Conjugate (H+L) is not recommended. As an alternative, use Perfect Protein Western Markers.*

3. Incubate for 60 min in 20 ml blocking solution.

Note: *PVDF or other hydrophobic membranes may require different blocking conditions (e.g. longer blocking times, higher concentrations of blocking reagent).*

4. Wash twice for 5 min each time with 20 ml 1X TBSTT.
5. Wash for 5 min with 20 ml 1X TBS.
6. Incubate for 1 h with 20 ml Nus•Tag Monoclonal Antibody diluted 1:10,000 in blocking solution.
7. Wash twice for 5 min each time with 20 ml 1X TBSTT.
8. Wash for 5 min with 20 ml 1X TBS.
9. Incubate for 1 h with 20 m l Goat Anti-Mouse IgG AP Conjugate in blocking solution.
10. Wash five times for 5 min each with 20 ml 1X TBSTT. It is important to thoroughly wash the membrane at this point to achieve maximum signal:noise ratios.

Note: *Washing steps should be performed in sufficient volume and repeated 5 times to assure complete removal of unbound conjugate. If background is evident, the blot can be washed several more times before adding additional substrate.*

The AP Detection Reagent Kit (Cat. No. 69264-3) contains enough NBT, BCIP and 20X AP Buffer for 25 blots (10 cm × 10 cm).

11. 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, toludinium salt in 100% dimethylformamide) with15 ml of 1X AP buffer (100 mM Tris, 100 mM NaCl, 1 mM $MgCl_2$, pH 9.5).
12. 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 signals should appear within 2–10 minutes.
13. To stop the reaction, wash the blot thoroughly in deionized water and allow to air dry. Store dry blots at room temperature wrapped in plastic.

Note: *Bacterial extracts contain endogenous 54.8 kDa NusA protein that may be detected.*

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Appendix A

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

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

References

1. Harrison, R.G. (2000) *in**Nova**tions* **11**, 4-7.
2. Davis, G.D., Elisee, C., Newham, D.M. and Harrison, R.G. (1999) *Biotechnol. Bioeng.* **65**, 382-388.