GST•Tag™ Monoclonal Antibody

About the Kit

GST•Tag™ Monoclonal Antibody	50 µg	71097-3
	250 µg	71097-4

Description

The GST•Tag™ Monoclonal Antibody is a mouse monoclonal antibody (IgG_1) with affinity for the 26 kDa glutathione-S-transferase (GST) domain from *S. japonicum*. This highly purified antibody is superior for detecting GST•Tag fusion proteins expressed in *E. coli*, yeast, mammalian, and *in vitro* transcription/translation systems by Western blotting, immunoprecipitation or immunofluorescence. GST•Tag fusion proteins are efficiently expressed in *E. coli* using Novagen's pET-41a-c(+), pET-42a-c(+), or pET-49b(+) vectors. The 50 µg GST•Tag Monoclonal Antibody kit provides enough antibody for 50 Western blots (10 × 10 cm).

Specificity	glutathione-S-transferase (GST) protein, precise epitope not determined
Species/isotype	Mouse monoclonal IgG_1
Cross-reactivity	Negligible with bacterial, insect or mammalian cell lysates
Sensitivity	2.5–5 ng: Western blot developed with chromogenic substrates
	<1 ng: Western blot developed with chemiluminescent substrate
Form	Stabilized solution of antibody in 50% glycerol
Working dilution	1:10,000 for Western blotting and immunofluorescence

Components

- 50 µg or 250 µg GST•Tag™ Monoclonal Antibody

Storage

Store GST•Tag Monoclonal Antibody at –20°C.

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Western Blotting

Chemiluminescent detection

This protocol is for transfer and detection of proteins on nitrocellulose membranes. Alkali-soluble Casein is the recommended blocking reagent. BSA or gelatin can be used for greater sensitivity, but may result in higher background.

Accurate size markers are recommended. Perfect Protein™ Western markers may be detected with S-protein HRP or AP Conjugate (see User Protocol TB102), or with His•Tag® Monoclonal Antibody (see TB283) followed by AP- or HRP-conjugated secondary antibody. Trail Mix™ Western Markers may be detected with S-Protein Conjugate (AP or HRP); see User Protocol TB310.

Preparation – per 10 × 10 cm blot

1. Prepare 120 ml fresh blocking solution. Either dilute 5% Alkali-Soluble Casein (Cat. No. 70955-3) to 1% with deionized water, or prepare Alkali-Soluble Casein stock (Cat. No. 218680) as per Appendix A (p 6). Alternatively, use 1% Gelatin or 3% BSA in 1X TBST (150 mM NaCl, 10 mM Tris HCl, 0.1% TWEEN®-20, pH 7.5).
2. Prepare 40 ml fresh blocking solution for primary and secondary antibody dilutions. We recommend 0.5% Alkali-Soluble Casein (5% Alkali-Soluble Casein (Cat. No. 70955-3) diluted to 0.5% in deionized water). Alternatively, prepare 0.5% Gelatin or 3% BSA in 1X TBST.
3. Prepare 80 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, 0.05% v/v TWEEN®-20, 0.2% v/v Triton® X-100, pH 7.5).
4. Dilute GST•Tag™ Monoclonal Antibody 1:10,000 (2 µl in 20 ml blocking solution).
5. Dilute Goat Anti-Mouse IgG HRP Conjugate (Cat. No. 71045-3) 1:5,000 (4 µl in 20 ml blocking solution). (For western blots, recommended range is 1:5000–1:10,000.)

Protocol

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

1. Run GST•Tag sample on an SDS-PAGE gel. Load protein markers in an adjacent lane.

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

2. Transfer proteins to membrane. Any Western transfer device can be used according to the manufacturer's instructions. Standard transfer buffer is 192 mM glycine, 25 mM Tris Base, 20% methanol, pH 8.3. If using Perfect Protein or Trail Mix Western markers, 150 and 225 kDa bands may transfer incompletely due to their large size. The 15 kDa band may not bind efficiently to membrane (particularly 0.45 micron nitrocellulose) due to its small size.
3. Wash membrane twice for 5 min each with 20 ml 1X TBS.
4. Incubate for 30 min in 20 ml blocking solution.

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

5. Wash twice for 5 min each using 20 ml 1X TBSTT.
6. Wash for 5 min with 20 ml 1X TBS.
7. Incubate for 1 h with 20 ml GST•Tag Monoclonal Antibody (1:10,000 in blocking solution).
8. Wash twice with 20 ml for 5 min each using 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 blocking solution.

Note: *HRP conjugates are not compatible with sodium azide. NaN_3 inhibits HRP activity.*

11. Wash 5 times for 5 min each with 20 ml 1X TBSTT. Thorough washing is critical.

Note: Washes should be performed in sufficient volume and repeated 5 times to remove unbound conjugate completely. If background is high, the blot can be washed several more times before adding more substrate.

12. After final washing step is complete, drain excess TBSTT from membrane by touching edge to a paper towel. Place membrane protein-side up in a clean tray or on plastic wrap.
13. For a 10 × 10 cm blot, use 1–1.5 ml chemiluminescent substrate working solution. Prepare substrate immediately before use. Wet entire surface of membrane with substrate and incubate blot at room temperature for 1 min.

Note: CDP-Star® AP Substrate (Cat. No. 69086-3) or SuperSignal® HRP Substrate (Cat. No. 69059-3) are available for sensitive chemiluminescent detection. Use 1.5 ml CDP-Star AP Substrate or 1 ml SuperSignal HRP Substrate. Prepare SuperSignal Substrate working solution by mixing equal parts 2X Luminol/Enhancer and 2X Stable Peroxide Solution.

14. Remove membrane from substrate. Drain excess substrate from membrane by touching the edge to a paper towel. Place membrane in a Development Folder (Cat. No. 69137-3) or on a fresh sheet of plastic wrap. Fold plastic over the membrane. Remove any bubbles between plastic and membrane. Gently remove any liquid from exterior of plastic.
Optional: Place a gLOCATOR™ Luminescent Label (Cat. No. 69102-3) on a corner of the Development Folder. Blot-identifying data may be written on the label for future reference.
15. Place membrane in a cassette with autoradiographic film. Expose for 1–10 min. An initial exposure time of 1 min is recommended. Longer exposures can be performed, though highest light output occurs in the first 5 min. Light output continues over several hours.

Colorimetric detection

This protocol is for transfer of proteins and detection on nitrocellulose membranes. BSA or gelatin are recommended blocking reagents. Alkali-soluble Casein (1%) may reduce background, but it may also decrease sensitivity.

Accurate size markers are recommended. Perfect Protein™ Western markers may be detected with S-protein HRP or AP Conjugate (see User Protocol TB102), or with His•Tag® Monoclonal Antibody (see TB283) followed by AP- or HRP-conjugated secondary antibody. Trail Mix™ Western Markers may be detected with S-Protein Conjugate (AP or HRP); see User Protocol TB310.

Preparation – per 10 × 10 cm blot

1. Prepare 20 ml fresh blocking solution. Use 1% BSA in 1X TBS (150 mM NaCl, 10 mM Tris-HCl, pH 7.5).
2. Prepare 40 ml fresh blocking solution for diluting primary and secondary antibodies. Use 1% BSA in 1X TBS.
3. Prepare 80 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, 0.05% v/v Tween®-20, 0.2% v/v Triton® X-100, pH 7.5).
4. Dilute GST•Tag™ Monoclonal Antibody 1:10,000 (2 µl into 20 ml blocking solution).
5. Dilute Goat Anti-Mouse IgG AP Conjugate (Cat. No. 69266-3) 1:5,000 in blocking solution (4 µl into 20 ml blocking solution).

Protocol

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

1. Run GST•Tag fusion protein sample on an SDS-polyacrylamide gel. Load protein markers in an adjacent lane.

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

2. Transfer proteins to membrane. Any Western transfer device can be used according to the manufacturer's instructions. Standard transfer buffer is 192 mM glycine, 25 mM Tris Base, 20% methanol, pH 8.3. If using Perfect Protein™ or Trail Mix™ Western markers, 150 and 225 kDa bands may transfer incompletely due to their large size. The 15 kDa band may not bind efficiently to membrane (particularly 0.45 micron nitrocellulose) due to its small size.
3. Wash membrane twice for 5 min each with 20 ml 1X TBS.
4. Incubate for 30 min in 20 ml blocking solution.

Note: PVDF or other hydrophobic membranes may require different blocking conditions (e.g., longer blocking, increased concentration of blocking reagent).

5. Wash twice for 5 min each with 20 ml 1X TBSTT.
6. Wash for 5 min with 20 ml 1X TBS.
7. Incubate for 1 h with 20 ml GST•Tag Monoclonal Antibody diluted 1:10,000 in blocking solution.
8. Wash twice for 5 min each 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 in blocking solution.
11. Wash 5 times for 5 min each with 20 ml 1X TBSTT. Thorough washing is critical.

Note: Washes should be performed in sufficient volume and repeated 5 times to remove unbound conjugate completely. If background is high, the blot can be washed several more times before adding more substrate.

12. Prepare developing solution. For each 10 × 10 cm blot , mix 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 (toluidine salt) in 100% dimethylformamide) in 15 ml 1X AP buffer (100 mM Tris, pH 9.5, 100 mM NaCl, 1 mM $MgCl_2$)

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

13. Place membrane protein-side up in a clean tray. Add developing solution. Incubate membrane at room temperature until purple color develops. Strong purple signal should appear within 2–10 minutes.
14. To stop the reaction, wash blot thoroughly in deionized water. Allow to air dry. Store dry blots at room temperature wrapped in plastic.

Immunoprecipitation Protocol

GST•Tag™ proteins can be isolated from cell extracts or *in vitro* transcription/translation reactions using the following protocol. GST•Tag Monoclonal Antibody is a mouse IgG_1 and binds strongly to Protein G. Resin choices include Protein G Plus Agarose (Cat. No. IP08), Protein G Plus/Protein A Agarose (Cat. No. IP10), and MagPrep® Anti-Mouse IgG Beads (Cat. No. 70996-3). MagPrep Beads are immobilized using a Magnetight™ (Cat. No. 70747-3) or similar magnetic stand.

Accurate size markers are recommended. Perfect Protein™ Western markers may be detected with S-protein HRP or AP Conjugate (see User Protocol TB102), or with His•Tag® Monoclonal Antibody (see TB283) followed by AP- or HRP-conjugated secondary antibody. Trail Mix™ Western Markers may be detected with S-Protein Conjugate (AP or HRP); see User Protocol TB310.

GST•Tag™ Antibody will be released from resin during SDS-PAGE. For non-radioactively labeled samples, subsequent immunoblot analysis with anti-mouse antibodies may detect the light (25 kDa) and heavy (50 kDa) chains. Target proteins of similar size may be obscured. Alternatively, antibodies may be covalently coupled to beads for use in immunoprecipitation (Cat. No. 400995.)

1. Consult the manufacturer's guidelines for preparation and binding capacities of Protein G resin. Prepare sample containing GST•Tag fusion protein.
   a. For *in vitro* transcription/translation reactions: Combine reaction with 0.5 ml 1X wash buffer (150 mM NaCl,20 mM Tris-HCl, 0.5% NP-40, 5 mM EDTA, 2 mM methionine, pH 8.0).
   b. For cell lysates: Prepare lysate in PBS or similar buffer. The following protocol is based on ~2–5 × 10^7cells. CytoBuster™ Protein Extraction Reagent (Cat. No. 71009-3) enables lysate preparation from mammalian and insect cells, and is specifically formulated for use in immunoprecipitation and Western blotting.

Note: Include negative control(s) (e.g., no antibody, no cell lysate, lysate from uninduced cells).

2. Pre-incubate sample with Protein G resin and (optionally) 1 µg of a normal mouse IgG. Pre-incubation and subsequent centrifugation will remove non-specifically bound proteins.
   a. For *in vitro* transcription/translation samples: Use 50 µl Protein G resin. Vortex, incubate on ice for 5 min, centrifuge at 16,000 × *g* for 3 min, and transfer supernatent.
   b. For cell lysates: Use 50 µl agarose and 5 µg normal mouse IgG per 1 ml cell lysate. Incubate for 40 min at 4°C on a rotating shaker. Centrifuge at 16,000 × *g* for 2 min and transfer supernatant to a new tube.
3. Add GST•Tag Antibody to the sample. Incubate for 1 h at 4°C on a rotating shaker.
   a. For *in vitro* transcription/translation reaction samples: add 1 µl GST•Tag Antibody.
   b. For cell lysates: add GST•Tag Monoclonal Antibody to a final concentration of 1–5 µg/ml. The ratio of antibody to GST•Tag fusion protein can be adjusted as needed.
4. Add Protein G Agarose to the sample, mix well and incubate for 1–2 h at 4°C. Calculate the appropriate amount to add based on the manufacturer's instructions.
5. Centrifuge at 16,000 × *g* for 2 min. Remove supernatant.
6. **a.** For *in vitro* transcription/translation samples: Add 0.75 ml 1X wash buffer (150 mM NaCl, 20 mM Tris HCl, 0.5% NP-40, 5 mM EDTA, 2 mM methionine, pH 8.0).
   b. For cell lysates: Add 100 µl PBS (137 mM NaCl, 4.3 mM Na_2HPO_4, 2.7 mM KCl, 1.47 mM KH_2PO_4, pH 7.3) and resuspend.
7. Centrifuge at 16,000 × *g* for 2 min. Transfer supernatant to a fresh tube.
8. Repeat Steps 6 and 7 twice, for a total of 3 washes.
9. Remove as much supernatant as possible. Resuspend final pellet in SDS sample buffer (e.g., Cat. No. 70607-3).
   a. For *in vitro* transcription/translation samples: Add 50 µl 2X SDS sample buffer to pellet.
   b. For cell lysates: Add 20–50 µl 2X SDS sample buffer.
10. Heat for 3 min at 85°C, cool to room temperature, centrifuge briefly, and load supernatant on an SDS-PAGE gel. Avoid agarose when pipetting sample.
11. Analyze the sample.
    a. For *in vitro* transcription/translation reactions (^{35}S-Methionine-labeled): fix gel by soaking in 10% TCA or isopropanol:water:acetic acid (25:65:10) for 20 min. Dry the gel and expose X-ray film. Optimal exposure time will vary based on ^{35}S Met incorporation and starting sample volume. Sensitivity can be increased ~10-fold with fluorography. Prior to drying, soak gel in scintillant solution (e.g., Amplify™) according to the manufacturer's instructions. Use intensifying screens during exposure. Strong signal should be observed under the following conditions:
       - 0.2–2 µg RNA template and 10–20 µCi of >600 Ci/mmol ^{35}S-Met in a 25 µl translation reaction
       - 10 µl translation mix per immunoprecipitation reaction and 14–20 h exposure.
    b. For non-radioactively labeled samples: perform a standard Western blot.

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

Immunofluorescence

We recommend the following protocol as a starting point. This protocol has been verified with COS-1 and CHO-K1 cells using Cy®-5 and Cy®-3 fluorophore-labeled secondary antibodies.

1. Prepare transfected cell cultures on coverslips or slide chambers at 1.5×10^5 cells/cm^2. Prepare 1X PBS (137 mM NaCl, 4.3 mM Na_2HPO_4, 2.7 mM KCl, 1.47 mM KH_2PO_4, pH 7.3) and PBSTB (1X PBS with 0.1% Triton X-100 and 0.2% BSA).
2. Remove medium and wash cells with 1X PBS for 5 min. Remove PBS.
3. Fix cells with freshly prepared 4% paraformaldehyde in 1X PBS for 15 min at room temperature.
4. Wash 2 times for 5 min each with 1X PBS at room temperature.

Note: At this point, the coverslips or slide chambers may be stored for several days at 4°C.

5. Permeabilize cells with 0.3% Triton® X-100 in 1X PBS for 10 min at room temperature. Handle cells carefully.
6. Wash 2 times for 5 min each with 1X PBS at room temperature.
7. Block with 2% BSA and 1% Horse Serum in 1X PBS for 20 min at room temperature.
8. Incubate with GST•Tag™ Monoclonal Antibody (diluted 1:10,000 in PBSTB) for 1 h at 37°C in a humid chamber.
9. Wash 2 times for 5 min each with PBSTB at room temperature.
10. Incubate with an appropriate secondary antibody diluted in PBSTB for 30 min at 37°C.
11. Wash 2 times for 5 min each with PBSTB at room temperature.
12. Remove PBSTB and stain nuclei with 1 µg/ml Hoechst 33258 (Calbiochem Cat. No. 382061) for 2 min at room temperature.
13. Wash 4 times for 5 min each with 1X PBS (no Triton X-100 or BSA) at room temperature.
14. Mount cells with mounting medium. Place one drop of mounting solution in each well or chamber of the slide or coverslip. Cover slowly with a slide or coverslip. Avoid introducing air bubbles. Seal edges with clear nail polish and store in darkness until polish has dried. Use appropriate fluorescent filter during microscopic analysis.

Appendix A

To prepare 5% Alkali-soluble Casein in 5X TBS from dry powder (Cat. No. 218680):

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