For life science research only.
Not for use in diagnostic procedures.

Anti-His_6
from mouse IgG_1 (clone BMG-His-1)

Version: 08
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Mouse monoclonal antibody for the detection of His_6-tagged recombinant proteins.
Lyophilized, stabilized

Cat. No. 11 922 416 001 100 µg

Store the lyophilizate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Content
1	Anti-His_6	1 vial, 100 µg

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the lyophilizate is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage/Stability
1	Anti-His_6	Store at +2 to +8°C.

Reconstitution

1. Add 1 ml double-distilled water to the lyophilizate to a final concentration of 100 µg IgG/ml.

2. Store 3 months at +2 to +8°C or aliquot and store at −15 to −25°C.

⚠ ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

For preparation of lyophilizate

- Double-distilled water

For western blotting

See section, **Working Solution** *for additional information on preparing solutions.*

- BSA* or Western Blocking Reagent*
- TBS
- Tween 20*
- BM Chemiluminescence Western Blotting Substrate (POD)*
- PVDF Membranes*
- Substrate for colorimetric detection (optional)

For immunoprecipitation

- Microcentrifuge
- Lysis buffer such as RIPA buffer: 50 mM Tris-HCl*, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF*, 1 g/ml Leupeptin*, 5 g/ml Aprotinin*, 1% Nonidet P-40*, 0.5% sodium deoxycholate, 0.1% SDS*
- Protein G Agarose*

For ELISA

- 50 mM sodium carbonate, pH 9.6
- BSA* or Blocking Reagent for ELISA*

1.4. Application

Anti-His_6 allows specific and sensitive detection of histidine-tagged proteins irrespective of the expression system used. It combines the advantages of efficient purification of histidine-tagged proteins by metal chelate affinity chromatography with the specific and sensitive detection of those proteins to study their function in numerous applications such as:

- Immunoblotting, such as dot blots and western blots
- Immunoprecipitation
- Immunoassays (ELISA)
- Immunocytochemistry
- Immunofluorescence

2. How to Use this Product

2.1. Before you Begin

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Preparation/Composition	Storage and Stability	For use in...
TBS (50 mM Tris, 150 mM NaCl)	• Dissolve 6.05 g Tris base* (50 mM) and 8.76 g NaCl (150 mM) in 800 ml PCR-grade water*. • Adjust pH to 7.5 with approximately 9.5 ml 1 M HCl • Dilute up to 1 l total volume with double-distilled water. Do not use sodium azide as an antimicrobial agent as it inhibits POD.	Store 3 months at +2 to +8°C.	Blocking and washing solutions.
TBST	Dilute 1 ml Tween 20* to 0.1% (v/v) final concentration in 1 l TBS.		Wash solution

2.2. Protocols

Western blotting

For optimal results, use uncharged membranes such as PVDF Western Blotting membranes*; Anti-His_6 recognizes a charged epitope, therefore charged transfer membranes can result in nonspecific binding of the antibody to the membrane.

1. Perform electrophoresis and transfer of the proteins to an appropriate membrane.
2. Block membrane with TBS containing 1% BSA*, casein, or Western Blocking Reagent*.
3. Incubate the blot with 0.2 to 0.5 µg/ml Anti-His_6 in TBS containing 1% BSA or Western Blocking Reagent for 1 hour at +15 to +25°C.
4. Wash 3 × 5 minutes with TBS containing 0.1% Tween 20 (TBST).
5. Incubate blot with an anti-mouse-peroxidase conjugate in TBS containing 1% BSA or Western Blocking Reagent for 1 hour at +15 to +25°C.
6. Wash 3 × 5 minutes with TBST.
7. Detect bound antibody for high sensitive detection with a chemiluminescence substrate such as BM Chemiluminescence Western Blotting Substrate (POD)* or use a precipitating substrate such as BM Blue POD Substrate, precipitating for colorimetric staining (Fig. 1).

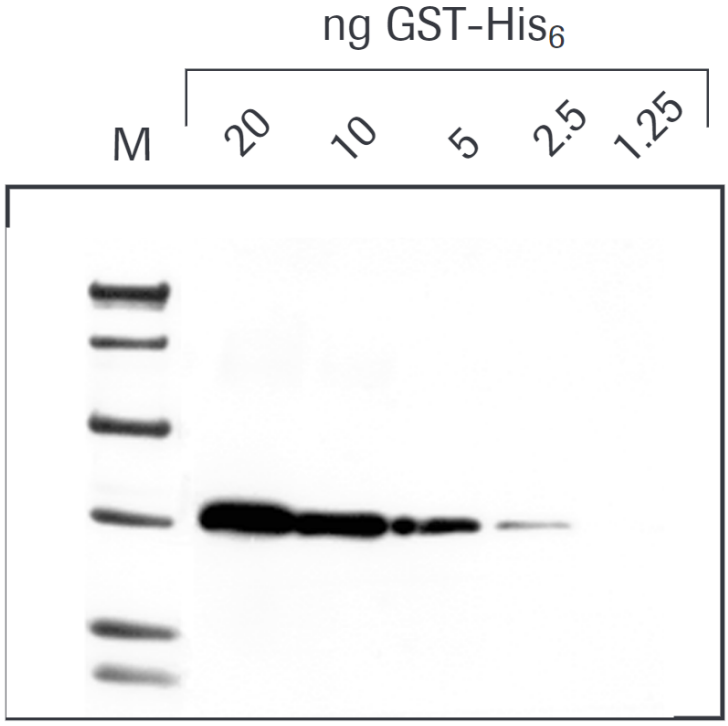

Fig. 1: Immunoblot of a His-tagged GST fusion protein (GST-His_6) serially diluted in an untransformed *E. coli* crude extract (2 µg total protein per lane) and indirectly detected with anti-mouse-POD (20 mU/ml) using the BM Chemiluminescence Western Blotting Substrate (POD)*. The concentration of Anti-His_6 was 500 ng/ml. M: Multi-Tag Marker.

Immunoprecipitation

See section, **Additional Equipment and Reagents Required** *for additional information on preparing solutions.*

1. Lyse cells with an appropriate Lysis buffer for 30 minutes on ice.
2. Centrifuge for 5 minutes in a microfuge at maximum speed.
   – Transfer supernatant to a new reaction vial.
3. Preclear the supernatant with Protein G Agarose* for 1 to 3 hours or overnight at +2 to +8°C.
4. Centrifuge for 1 minute in a microfuge at maximum speed.
   – Transfer supernatant to a new reaction vial.
5. Add Anti-His_6 to the supernatant to a final concentration of 0.5 to 5 µg/ml.
6. Incubate 1 to 3 hours or overnight at +2 to +8°C.
7. Add Protein G Agarose to collect the immune complex.
   – Incubate for 1 to 3 hours at +2 to +8°C.
8. Wash beads thoroughly with Lysis buffer before further analysis.

ELISA

Anti-His_6 can be used as a capture or detection antibody.

Capture antibody

1. Use 1 to 5 µg/ml IgG in 50 mM sodium carbonate, pH 9.6, for coating.
2. Incubate 100 µl/well in a 96-well plate for 2 hours at +15 to +25°C or overnight at +2 to +8°C.

Detection antibody

1. Incubate antibody at +15 to +25°C for 1 hour.

For best results, use an antibody concentration of 100 ng/ml in TBS containing 1% BSA or Blocking Reagent for ELISA.*

2.3. Parameters

Specificity

Anti-His_6 specifically recognizes an epitope of six consecutive histidine residues of both natural and recombinant proteins. The antibody reacts with native and denatured histidine-tagged fusion proteins independent of the location of the histidine-tag in the protein sequence.

3. Additional Information on this Product

3.1. Test Principle

Background information

For the purification of large amounts of recombinant proteins, several strategies have been developed, including the:

- Expression of fusion proteins, allowing the fused protein to be purified by affinity chromatography. As most of the fusion partners, such as glutathione-S-transferase or protein A are large proteins, there is a potential risk that they alter the properties of the protein of interest. Fusion proteins must be expressed in a native form for efficient isolation after affinity chromatography.
- Addition of a stretch of six consecutive histidine residues to the protein. The affinity of the His-tag for metal ions allows the tagged protein to be separated from other proteins in the crude cell extract with high purity using metal chelate affinity chromatography. Fusing only six residues to the protein also minimizes the risks of altering the properties of the protein of interest. Purification under denaturing conditions is also possible.

Several antibodies and chelator/enzyme conjugates have been reported to detect histidine-tagged proteins. However, these are restricted to specific expression vectors or recognize histidine-tagged proteins with only moderate affinity.

Preparation

1. Anti-His_6 was obtained by immunizing mice with a mixture of histidine-tagged proteins.
2. Spleen cells were isolated and fused with P3-X63-Ag8.653 myeloma cells by standard methods.
3. Hybridoma supernatants were screened for specific binding to histidine-tagged fusion proteins.
4. Hybridomas secreting monoclonal antibodies specific for the His_6-epitope were isolated and cloned by limiting dilution.
5. The antibody was purified from bioreactor supernatants by adsorption chromatography and lyophilized in the presence of proteinous stabilizers.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tris base	1 kg, *Not available in US*	10 708 976 001
	1 kg	03 118 142 001
	5 kg	11 814 273 001
PVDF Western Blotting Membranes	1 roll, 30 cm x 3.00 m	03 010 040 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Western Blocking Reagent, Solution	100 ml, 10 blots, 100 cm^2	11 921 673 001
	6 x 100 ml, 60 blots, 100 cm^2	11 921 681 001
Blocking Reagent	27 g, for one liter blocking solution, *Not available in US*	11 112 589 001
BM Chemiluminescence Western Blotting Substrate (POD)	1 set, 1,000 cm^2 membrane (trays), 6,250 cm^2 membrane (transparent plastic bags)	11 500 708 001
	1 set, 4,000 cm^2 membrane (trays), 25,000 cm^2 membrane (transparent plastic bags)	11 500 694 001
Protein Agarose	Protein G Agarose, 2 ml	11 719 416 001
	Protein A Agarose, 2 ml	11 719 408 001
	Protein G Agarose, 5 ml	11 243 233 001
	Protein A Agarose, 5 ml	11 134 515 001
	Protein G Agarose, 15 ml, *Not available in US*	05 015 952 001
	Protein A Agarose, 15 ml, *Not available in US*	05 015 979 001
Bovine Serum Albumin Fraction V	50 g	10 735 078 001
	100 g, *Not available in US*	10 735 086 001
	500 g, *Not available in US*	10 735 094 001
	1 kg, *Not available in US*	10 735 108 001
Tris hydrochloride	500 g	10 812 846 001
PMSF	10 g	10 837 091 001
	25 g	11 359 061 001
Leupeptin	5 mg	11 017 101 001
	25 mg	11 017 128 001
	50 mg	11 034 626 001
	100 mg	11 529 048 001
Aprotinin	10 mg	10 236 624 001
	50 mg	10 981 532 001
	100 mg	11 583 794 001
Nonidet P-40 Substitute	100 ml	11 754 599 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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