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

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

Version: 08
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Mouse monoclonal antibody for the detection of histidine-tagged recombinant proteins, conjugated with peroxidase.
Lyophilized

Cat. No. 11 965 085 001 50 U

Store the lyophilizate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Anti-His_6-Peroxidase	▪ White lyophilizate ▪ Lyophilized in the presence of proteinous stabilizers.	1 vial, 50 U

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
1	Anti-His_6-Peroxidase	Store at +2 to +8°C.

Reconstitution

1. Add 1 ml double-distilled water to the lyophilizate to a final concentration of 50 U/ml.
   – Let stand for at least 10 minutes at +15 to +25°C.

2. Mix thoroughly; do not vortex.

3. Store 2 months at +2 to +8°C or aliquot and store 6 months at −15 to −25°C.

⚠ ***Avoid repeated freezing and thawing; this will affect the peroxidase activity.***
⚠ ***Do not add sodium azide as a preservative because it inhibits the activity of the peroxidase.***

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.*

- PBS*
- Double-distilled water
- PVDF Western Blotting Membranes*
- Tween 20*
- BM Chemiluminescence Blotting Substrate (POD)*
- Blocking Reagent*
- Na_2HPO_4, analysis grade
- NaH_2PO_4, analysis grade

For ELISA

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

- PBS*
- Double-distilled water
- Microplates, such as Nunc Maxisorp
- Microplate washer (optional)
- Microplate reader
- Tween 20*
- Blocking Reagent*
- BM Blue POD Substrate, soluble*
- Sodium carbonate, analysis grade
- Sulfuric acid, 95 to 97%, analysis grade

1.4. Application

Anti-His_6-Peroxidase can be used for the following applications:

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

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

Western blotting

Solution	Composition/ Preparation	Storage and Stability	For use in...
Phosphate buffered saline (PBS), 10x	100 mM phosphate, 1.5 M NaCl, pH 7.2	Store 1 week at +2 to +8°C or at least 2 years at −15 to −25°C.	Preparation of 1x PBS.
PBS, 1x	Dilute 10 ml 10x PBS with double-distilled water to a final volume of 100 ml.		▪ Preparation of blocking solution. ▪ Washing solution ▪ Anti-His_6-Peroxidase solution
Washing solution	1x PBS, containing 0.1% Tween 20* (v/v).	Store 1 week at +2 to +8°C.	Washing
Blocking solution	1x PBS, containing 1% (w/v) Blocking Reagent*.	Store 1 week at +2 to +8°C or at least 2 years at −15 to −25°C.	Blocking
Anti-His_6-Peroxidase working solution	Dilute the reconstituted antibody 1:500 to 100 mU/ml using Blocking solution.	Unstable, prepare immediately before use.	Detection

ELISA

Solution	Composition/ Preparation	Storage and Stability	For use in...
Sodium carbonate solution	50 mM, pH 9.6	Prepare immediately before use.	Coating
Phosphate buffered saline (PBS), 10x	100 mM phosphate, 1.5 M NaCl, pH 7.2	Store 1 week at +2 to +8°C or at least 2 years at −15 to −25°C.	Preparation of 1x PBS.
PBS, 1x	Dilute 10 ml 10x PBS with double-distilled water to a final volume of 100 ml.		▪ Preparation of blocking solution. ▪ Washing solution ▪ Anti-His_6-Peroxidase solution
Washing solution	1x PBS, containing 0.1% Tween 20* (v/v).	Store 1 week at +2 to +8°C.	Washing
Blocking solution	1x PBS, containing 1% (w/v) Blocking Reagent*.	Store 1 week at +2 to +8°C or at least 2 years at −15 to −25°C.	Blocking
Coating solution	Dilute 1 to 10 µg of the appropriate protein in 1 ml Sodium carbonate solution.	Prepare immediately before use.	Coating
Anti-His_6-Peroxidase working solution	Dilute the reconstituted antibody 1:500 to 100 mU/ml using Blocking solution.	Unstable, prepare immediately before use.	Detection

2.2. Protocols

Western blotting

The following procedure describes the detection of a His_6-tagged protein by enzyme-mediated chemiluminescence. If using other detection systems, such as colorimetric, the conditions may need to be adapted. Since Anti-His_6-Peroxidase recognizes a charged epitope, charged transfer membranes can result in nonspecific binding of the antibody to the membrane. For best results, use uncharged membranes, such as PVDF Western Blotting Membranes*.

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

1. Perform electrophoresis and transfer the proteins to a PVDF membrane.
2. Block the membrane with Blocking solution for 1 hour at +15 to +25°C or overnight at +2 to +8°C.
3. Incubate the blot with 100 mU/ml Anti-His_6-Peroxidase solution for 1 hour at +15 to +25°C.
4. Wash 3 × 5 minutes with Washing solution.
5. Detect bound immunocomplexes with a chemiluminescence substrate as described in the Instructions for Use of the BM Chemiluminescence Blotting Substrate (POD)*.

The following picture shows a typical result regarding the specificity and sensitivity of the detection of histidine-tagged proteins by western blotting (Fig. 1).

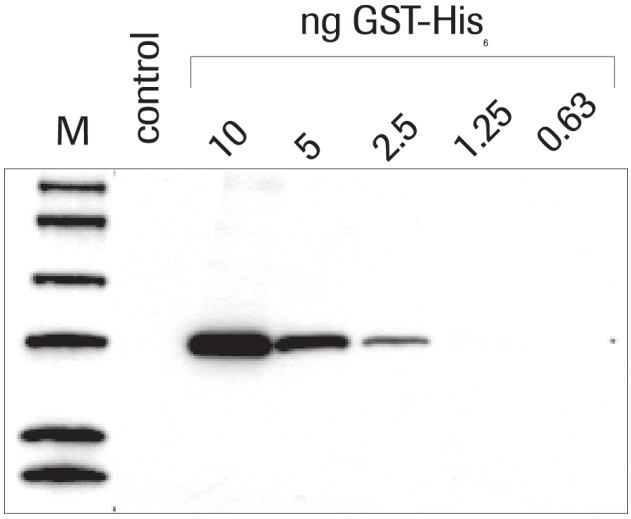

Fig. 1: Immunoblot of a His_6-tagged GST fusion protein (GST-His_6, 30 kD) serially diluted in an untransfected eukaryotic cell extract (20 µg total protein per lane) and directly detected using Anti-His_6-Peroxidase (100 mU/ml) and the BM Chemiluminescence Western Blotting substrate (POD)*. The control lane is an untransfected eukaryotic cell extract (20 µg total protein). M: Multi-Tag Marker, protein molecular weight markers, 10 kD to 100 kD.

ELISA

To avoid evaporation of the solutions, cover the plate with adhesive cover foils or special microplates during all incubation steps.

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

1. Coat wells with 100 µl/well Coating solution for 1 to 2 hours at +37°C or overnight at +2 to +8°C.
2. Wash 5 × with Washing solution, removing residual solution.
3. Add 300 µl Blocking solution per well.
   – Incubate for 1 to 2 hours at +37°C or overnight at +2 to +8°C.
4. Wash 5 × with Washing solution, removing residual solution.
5. Add 100 µl Anti-His_6-Peroxidase solution per well.
   – Incubate for 1 hour at +15 to +25°C.
6. Wash 5 × with Washing solution, removing residual solution.
7. Add 100 µl/well BM Blue POD Substrate, soluble*, prewarmed to +15 to +25°C.
   – Incubate at +15 to +25°C under constant shaking until the color development is sufficient.
8. To stop the color development, add 100 µl/well 2 M sulfuric acid.
9. Read the absorbance at 450 nm (reference wavelength: 690 nm) within 30 minutes after stopping the reaction.

2.3. Parameters

Specificity

Anti-His_6-Peroxidase 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 epitope-sequence location; however, it preferentially recognizes the C-terminal His_6 epitope with high sensitivity.

Working Concentration

Use the following working concentrations for each application.

- ELISA: 100 mU/ml
- Western and dot blot: 100 mU/ml

3. Troubleshooting

Observation	Possible cause	Recommendation
Nonspecific reactivity especially with high total protein loading.	Inadequate buffer conditions.	Use PBS containing Blocking Reagent for membrane blocking and dilution of the Anti-His_6-Peroxidase.
		Prolong time for membrane blocking.
Staining of the protein of interest is too weak or too strong.	Inadequate amounts of protein loaded onto the gel.	Increase or decrease the amount total protein loading.
	Inadequate conditions used for detection.	Increase or decrease the concentration of Anti-His_6-Peroxidase.
		Shorten or prolong exposure time used during detection.

4. Additional Information on this Product

4.1. Test Principle

Purification of recombinant proteins – conventional techniques

Recombinant proteins are frequently purified using an application that expresses the protein as a chimera with an easily purified fusion partner. This includes:

- Fusions with large polypeptides, such as glutathione-S-transferase (GST), or
- protein A.

This enables the fused protein to be purified by affinity chromatography.

Disadvantages of conventional techniques

Large proteins as fusion partners, however, increase the risk of altering the properties of the protein of interest. Additionally, the fusion protein has to be expressed in a native form for efficient isolation by affinity chromatography.

Epitope tagging – His_6

Another very successful approach for purification of fusion proteins is the addition of a stretch of six consecutive histidine residues (His_6-tag) to the protein. The affinity of the His_6-tag for metal ions allows the tagged protein to be separated from other proteins in a crude cell extract with high purity using metal chelate affinity chromatography. As there are only six histidines, the risks of altering the properties of the protein of interest are minimized. Additionally, purification under denaturing conditions is possible.

Anti-His_6 antibodies in epitope tagging

Several antibodies and chelator/enzyme conjugates have been reported to detect histidine-tagged proteins. However, these are restricted by requiring additional amino acids adjacent to the histidine sequence (requiring specific expression vectors) or recognize histidine-tagged proteins with only moderate affinity. Anti-His_6-Peroxidase is a monoclonal antibody directly conjugated to horseradish peroxidase which allows specific and sensitive detection of histidine-tagged proteins irrespective of the expression system used. Therefore, Anti-His_6-Peroxidase links the advantages of efficient purification of histidine-tagged proteins by metal chelate affinity chromatography with specific and sensitive detection of those proteins in numerous applications to study the function of these proteins (epitope tagging).

4.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

5. Supplementary Information

5.1. 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.

5.2. Changes to previous version

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

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
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
BM Blue POD Substrate, soluble	100 ml	11 484 281 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001

5.4. Trademarks

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

5.5. License Disclaimer

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

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

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

5.8. 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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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany