GE Healthcare
Life Sciences

Anti-His Antibody

Mouse anti-His Antibody from mouse ascites fluid.

Product Specification Sheet

Code: 27-4710-01

Warning
For research use only.
Not recommended or intended for diagnosis of disease in humans or animals.
Do not use internally or externally in humans or animals.

Storage

Store at -15°C to -30°C.

For greatest stability, **Anti-His Antibody** should be divided into single-use aliquots after thawing for its initial use and each aliquot stored at -15°C to -30°C or below. Repeated cycles of freeze/thaw may cause loss of activity and should be avoided.

Safety warnings and precautions

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Quality control

Anti-His Antibody is tested for its ability to detect 100 ng of a Glutathione S-transferase (GST)/His_6-fusion protein on a nitrocellulose Western blot. The blot is developed using the **Anti-His Antibody** at 1:3 000 dilution, peroxidase conjugated anti-mouse IgG and TMB (3,3',5,5'-tetramethylbenzidine) as substrate.

Components

Anti-His Antibody is a monoclonal antibody (IgG2a) that reacts with polypeptides which include a His_6 tag. The antibody preparaion is supplied as clarified mouse ascites fluid with 0.1% sodium azide as preservative. The antibody is provided unconjugated to allow maximum flexability in choice of detection systems. The **Anti-His Antibody** can be readily detected using secondary antibodies such as: anti-mouse IgG alkaline phosphatase conjugate [available from Sigma Chemical Company (Catalogue Number A-4312)], anti-mouse IgG peroxidase conjugate code NA931 available from GE Healthcare.

Total Protein (biuret): 20–40 µg/µl.

Mouse IgG2a Concentration (by Radial Immuno Diffusion, RID): 1.4–2.8 µg/µl.

Other materials required

General

- Anti-mouse secondary antibody for detection of **Anti-His Antibody**

For Western Blot

- Nitrocellulose membrane (e.g., Hybond™ ECL™ for code numbers see related products).
- 10× PBS: 1.4 M NaCl, 27 mM KCl, 101 mM Na_2HPO_4, 18 mM KH_2PO_4, pH 7.3.
- Blocking/incubation buffer: 10% (w/v) non-fat dry milk, 1x PBS, 0.05% Tween™-20.
- Wash buffer: 1× PBS, 0.05% Tween-20.
- Reagents for blot detection.

For ELISA

- 96-well microplate
- 10× PBS: 1.4 M NaCl, 27 mM KCl, 101 mM Na_2HPO_4, 18 mM KH_2PO_4, pH 7.3.
- Blocking/incubation buffer: 3% (w/v) non-fat dry milk, 1× PBS, 0.05% Tween-20.
- Wash buffer: 1× PBS, 0.05% Tween-20.
- Substrate for ELISA development.

Protocol

1. Detection of His-tagged Protein by Western Blot

Gel Fractionation and Membrane Transfer

- Fractionate proteins on an SDS polyacrylamide slab gel.
- Transfer the separated proteins from the gel to an appropriate membrane (e.g., Hybond ECL).

Blocking Membrane

- Transfer the membrane on to which the proteins have been blotted to an appropriate container (e.g., petri dish or small baking dish).
- Add 50–200 ml of blocking/incubation buffer (see Other materials required).
- Incubate for 1–16 hours at ambient temperature with gentle shaking.

  NOTE: Longer incubation times (up to 16 hours) with blocking/ Incubation buffer may reduce background signal.
- Decant and discard the buffer.

Anti-His Antibody binding

- Prepare a 1:3 000 dilution of **Anti-His Antibody** with blocking/ incubation buffer (see Other materials required) (e.g., 6.7 µl of antibody to
  20 ml of buffer).

  NOTE: The recommended dilution may not be optimal for all assays. If required, a titration of the **Anti-His Antibody** should be performed to determine the optimal dilution.
- Pour the antibody/buffer mixture into the container with membrane.
- Incubate for 1 hour at ambient temperature with gentle mixing.
- Decant and discard the antibody/buffer.

  NOTE: Longer incubations may improve sensitivity but may increase background.
- Wash the membrane with 20–30 ml of wash buffer for 5 minutes at ambient temperature with gentle mixing.
- Discard the wash buffer and repeat wash two additional times.

Binding of Secondary Antibody

- Dilute the secondary antibody (see above for examples) with blocking/incubation buffer (see other materials required) according to the manufacturer's recommendation.
- Pour the antibody/buffer mixture into the container with membrane.
- Incubate for 1 hour at ambient temperature with gentle mixing.
- Decant and discard the antibody/buffer.
- Wash the membrane with 20–30 ml of wash buffer for 5 minutes at ambient temperature with gentle mixing.
- Discard the wash buffer and repeat wash two additional times.
- Develop the blot with appropriate substrate for the conjugated second antibody.

2. Assay of His-tagged Protein by ELISA

- Transfer 50 µl of each His-tagged protein preparation to be assayed into a well of a 96-well multiwell plate (e.g., polystyrene for ELISA). Include a negative control (e.g., extract from untransformed cells) in the set of proteins to be assayed.
- Incubate the multiwell plate for 1 hour at room temperature in a humidified container (e.g., a sealed container containing moistened paper towels).
- Empty the contents of the multiwell plate wells. To do this, invert the plate and shake the well contents into a suitable container. If the antigen is a biohazard, pipette or aspirate the well contents into an appropriate biohazard receptacle. All solutions that come into contact with a plate coated with a biohazard are assumed to be contaminated.
- Tap the inverted plate on a paper towel to remove any liquid remaining in the wells.
- Completely fill each well of the multiwell plate with blocking/ incubation buffer, and incubate the multiwell plate for 30 minutes at room temperature as above.
- Empty the contents of the multiwell plate and blot it on a paper towel as before.
- Prepare a 1:1 000–1:6 000 dilution of **Anti-His Antibody** with blocking/incubation buffer (see Other materials required) (e.g., 3.3–20 µl of antibody to 20 ml of buffer).
- Add 100 µl of diluted **Anti-His Antibody** to each coated well (including those containing negative control) and incubate the plate for 1 hour at room temperature.
- Empty the contents of the multiwell plate and blot it on a paper towel.
- Wash the wells by filling each well with wash buffer. Invert the plate and shake out the contents. Repeat this process a total of four times.
- Dilute the secondary antibody (see above for examples) with blocking/incubation buffer (see Other materials required) according to the manufacturer's recommendation.
- Add 100 µl of diluted secondary antibody to the appropriate wells and incubate the plate for 1 hour at room temperature.
- Wash the wells by filling each well with wash buffer. Invert the plate and shake out the contents. repeat this process a total of four times.
- Develop the plate with the appropriate substrate for the secondary antibody.

Troubleshooting guide

Problem: Weak or no signal detectable on Western Blot.
Possible cause/solutions

A. His tagged fusion protein not expressed or expressed at very low levels. Check Coomassie™ stained gel of samples being analyzed for presence of band corresponding to His-tagged fusion protein to be detected.

B. His-tag not being expressed in fusion protein. Check sequence of fusion gene to ensure that sequences encoding the His-tag are present and in the proper translational reading frame.

C. Proteins not being transferred efficiently from gel to membrane. Use a pre-stained marker on electrophoresis gel and monitor transfer to membrane over entire size range.

D. Secondary antibody not binding to **Anti-His Antibody**. Make sure that secondary antibody is directed against mouse IgG. Use a different source of secondary antibody.

E. Low or no activity of enzyme conjugated to secondary antibody. Some conjugated secondary antibodies have limited lifetimes and strictly defined storage conditions. Check expiry of secondary antibody. Check activity of secondary antibody with different primary antibody if necessary. Use a different source of secondary antibody or a different conjugate.

F. Substrates for detection not functioning properly. Some substrates must be made freshly just prior to use. Test substrates in mock reaction. Make sure that all components are of high quality. Some substrates do not result in adequate signal strength. Try a different substrate.

Problem: High background on Western Blot
Possible cause/solutions

A. Blocking/incubation buffer not functioning. Make fresh blocking/incubation buffer. Make all dilutions of antibodies in blocking/incubation buffer. Allow blocking of membrane to take place for at least 1 hour with gentle shaking.

B. Secondary antibody is binding non-His- tagged sample proteins. Dilute secondary antibody to the proper concentration. Treat an identical Western blot with secondary antibody only (no **Anti- His Antibody**) followed by standard detection to determine background caused by secondary antibody. Use a secondary antibody that has been pre-adsorbed against *E.coli* proteins.

C. Sample proteins/extracts have been overloaded on gel. High protein concentration may increase non-specific binding of **Anti-his Antibody**. Reduce the amount of sample loaded.

Problem: Weak or no signal detectable in ELISA
Possible cause/solutions

A. His-tagged fusion protein not expressed or expressed at very low levels. Check Coomassie stained gel of samples being analyzed for presence of band corresponding to His-tagged fusion protein to be detected.

B. His-tag not being expressed in fusion protein. Check sequence of gene to ensure that sequences encoding the His-tag are present and in the proper translational reading frame.

C. Proteins not being bound efficiently to multiwell plate. Plates designed for ELISA having optimized binding characteristics for proteins should be used. Multiwell plates vary between suppliers. Use a different source of multiwell plate.

D. Secondary antibody not binding to **Anti-His Antibody**. Make sure that secondary antibody is directed against mouse IgG. Use a different source of secondary antibody.

E. Low or no activity of enzyme conjugated to secondary antibody. Some conjugated secondary antibodies have limited lifetimes and strictly defined storage conditions. Check expiry of secondary antibody. Check activity of secondary antibody with different primary antibody if necessary. Use a different source of secondary antibody or a different conjugate.

F. Substrates for detection not functioning properly. Some substrates must be made freshly just prior to use. Test substrates in mock reaction: bind secondary antibody to unblocked well, block, and perform standard detection scheme. Make sure that all components are of high quality. Some substrates do not result in adequate signal strength. Try a different substrate.

Problem: High background in ELISA
Possible cause/solutions

A. Blocking/incubation buffer not functioning. Make fresh blocking/incubation buffer. Make all dilutions of antibodies in blocking/incubation buffer. Allow blocking of multiwell plate to take place for at least 1 hour after initial protein binding. Make sure that wells are completely filled with blocking/incubation buffer.

B. Secondary antibody is binding to non-his- tagged sample proteins. Dilute secondary antibody to the proper concentration. Treat a sample well with secondary antibody only (no **Anti-His Antibody**) followed by standard detection to determine background caused by secondary antibody. Use a secondary antibody that has been pre-adsorbed against *E.coli* proteins.

C. Sample protein/extracts have been overloaded in the well. High concentration may increase non-specific binding of **Anti-His Antibody**. Reduce the amount of sample applied to well.

Related products*

His Buffer Kit	11-0034-00
His GraviTrap™	11-0033-99
His GraviTrap Kit	28-4013-51
His GraviTrap TALON®	29-0005-94
His MultiTrap™ HP	28-4009-89
His MultiTrap FF	28-4009-90
His MultiTrap TALON	29-0005-96
His Ni Sepharose™ Ni	28-9673-88 28-9673-90 28-9799-17
His Ni Sepharose excel	17-3712-20 17-3712-21 17-3712-22
His SpinTrap™	28-4013-53
His SpinTrap Kit	28-9321-71
His SpinTrap TALON	29-0005-93
HisTrap™ HP 5 × 1 ml	17-5247-01
HisTrap HP 5 × 5 ml	17-5248-02
HisTrap FF crude 5 × 1 ml	11-0004-58
HisTrap FF crude 5 × 5 ml	17-5286-01
HisTrap excel 5 × 1 ml	17-3712-05
HisTrap excel 5 × 5 ml	17-3712-06
HiTrap™ TALON crude 5 × 1 ml	28-9537-66
HiTrap TALON crude 5 × 5 ml	28-9537-67

Hybond ECL

9 × 10 cm	RPN910D
10 × 10 cm	RPN1010D
3 m × 30 cm	RPN303D
Hybond P	RPN2020F
ECL Western Blotting Detection Reagents	RPN2109
Amersham ECL Prime Western blotting detection reagent	RPN2232
Amersham ECL Select Western blotting detection reagent	RPN2235
ECL Mouse IgG, HRP-Linked Whole Ab (from sheep)	NXA931-1ML
Amersham ECL Mouse IgG, HRP-Linked Whole Ab (from sheep)	NA931-1ML
Amersham ECL Mouse IgG, HRP-Linked Whole Ab (from sheep)	NXA931-100µL
ECL Mouse IgG, HRP-Linked F (Ab') 2 Fragment (from sheep)	NA9310-1ML

Legal

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