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

Anti-HA High Affinity
from rat IgG$_1$

Version: 10
Content Version: December 2020

Rat monoclonal antibody (clone 3F10)
Lyophilized, stabilized

Cat. No. 11 867 423 001	50 µg
Cat. No. 11 867 431 001	500 µg

Store the lyophilizate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Catalog Number	Content
1	Anti-HA High Affinity, Rat monoclonal antibody (clone 3F10)	11 867 423 001	1 vial, 50 µg
		11 867 431 001	1 vial, 500 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
1	Anti-HA High Affinity	Store at +2 to +8°C.

Reconstitution

1. Add 0.5 ml (11 867 423 001; 50 µg) or 2.5 ml (11 867 431 001; 500 µg) double-distilled water to the lyophilizate to a final concentration of 100 µg/ml or 200 µg/ml, respectively.
2. Let stand 10 minutes at +15 to +25°C.
3. Mix thoroughly; do not vortex.
4. 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 Western Blotting Membranes*
- Streptavidin-POD*
- Anti-Rat-Ig-Biotin

For immunoprecipitation

- Microcentrifuge
- Lysis buffer, such as, 50 mM Tris-HCl*, pH 7.5; 150 mM NaCl; 1% Nonidet P-40*; 0.5% sodium deoxycholate; 0.7 µg/ml Pepstatin; 1 tablet cOmplete* protein inhibitor cocktail/50 ml or 1 tablet cOmplete Mini* protein inhibitor cocktail/10 ml; Store aliquoted at −15 to −25°C; mix carefully after thawing.
- Protein G Agarose*

For ELISA

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

1.4. Application

Anti-HA High Affinity allows specific and sensitive detection of native and recombinant HA-tagged proteins to study their function in numerous applications, such as:

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

Anti-HA High Affinity rat monoclonal antibody can be used in conjunction with murine monoclonal antibodies for double labeling.

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 double-distilled 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

Directly conjugated secondary antibodies, such as anti-rat peroxidase conjugate may be used successfully with Anti-HA High Affinity. However, with certain sample material, such as mammalian and yeast extracts, nonspecific reactivity has been observed which is due to the anti-rat secondary antibody and the total protein loading (>10 µg). Avoid it by using the indirect anti-rat biotin/streptavidin system and loading <10 µg total protein.

i *For detection of HA-tagged proteins in western blots via alkaline phosphatase or peroxidase, use Anti-HA High Affinity and the appropriate conjugated secondary antibody, such as Anti-rat Ig-Alkaline Phosphatase or Anti-rat Ig-Peroxidase together with the corresponding detection system.*

1. Perform electrophoresis and transfer of the proteins to an appropriate membrane.

2. Block membrane with TBS containing 1% BSA*, casein, or Blocking Reagent*.
   i *When loading the gel, use <10 µg total protein.*

3. Incubate the blot with 50 to 200 ng/ml Anti-HA High Affinity 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 Anti-Rat-Ig-Biotin secondary antibody according to the instructions of the supplier, for example, TBS containing 1% BSA or Western Blocking Reagent, for 30 minutes at + 15 to + 25°C.

6. Wash 3 × 5 minutes with TBST.

7. Incubate the blot with 5 to 15 mU/ml Streptavidin-POD in TBS containing 1% BSA or Western Blocking Reagent for 30 minutes at +15 to +25°C.

8. Detect bound antibody for high sensitive detection with a chemiluminescence substrate, such as BM Chemiluminescence Blotting Substrate (POD)* (Fig. 1).

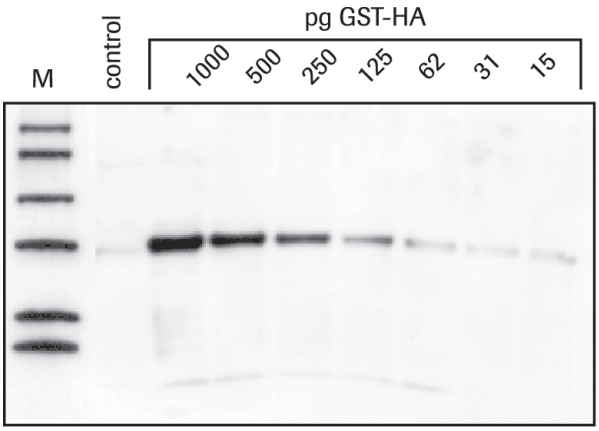

Fig. 1: Immunoblot of a HA-tagged GST fusion protein (GST-HA) serially diluted in an untransfected eukaryotic cell extract (10 µg total protein per lane) and indirectly detected using Anti-Rat-Ig-Biotin and Streptavidin-POD* (10 mU/ml) using BM Chemiluminescence Western Blotting substrate (POD)*. The concentration of the Anti-HA High Affinity was 100 ng/ml. The control lane is an untransfected eukaryotic cell extract (10 µg total protein). 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-HA High Affinity to the supernatant to a final concentration of 0.5 to 5 µg/ml.
   – Incubate 1 to 3 hours or overnight at +2 to +8°C.

6. Add Protein G Agarose to collect the immune complex.
   – Incubate for 1 to 3 hours at +2 to +8°C.

7. Wash beads thoroughly with Lysis buffer before further analysis.

ELISA

Anti-HA High Affinity 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-HA High Affinity (3F10) specifically recognizes the HA peptide sequence [YPYDVPDYA] derived from the influenza hemagglutinin protein. The antibody recognizes its antigenic determinant even when the HA peptide epitope is introduced into unrelated recombinant proteins by a technique known as epitope tagging.

Working Concentration

Use the following working concentrations for each application.
- ELISA: for detection, 100 ng/ml; for coating, 1 to 5 µg/ml
- Immunoprecipitation: 0.5 to 5 µg/ml
- Western and dot blot: 50 to 200 ng/ml

3. Additional Information on this Product

3.1. Test Principle

Background information

The Anti-HA High Affinity antibody (clone 3F10) recognizes the same epitope as clone 12CA5, which was originally used to study how the immune system recognizes the influenza hemagglutinin protein, a surface glycoprotein required for infectivity of the human virus. However, the principal use of the Anti-HA antibody is the detection and purification of proteins whose encoding DNA sequences have been fused to the HA epitope sequence by recombinant techniques, that is epitope tagging. The ability to prepare such epitope-tagged proteins and locate them with the Anti-HA antibody in subsequent experiments has enabled researchers to determine:

- The size, cellular localization, and abundance of proteins produced by newly discovered genes.
- Post-translational modifications of proteins.
- The movement of proteins within cell membranes.
- The identity of proteins within functional protein complexes.
- The function of proteins that are unstable, difficult to purify, or share epitopes with a number of other proteins.

However, cross-reacting bands have been reported in certain western blot experiments using Anti-HA 12CA5. Anti-HA High Affinity is a monoclonal antibody whose high affinity and low working concentration result in less cross-reactivity when compared with other antibodies to the HA-epitope.

Preparation

1. Anti-HA High Affinity was obtained by immunizing mice with a synthetic peptide (residues 76-111 of X47 hemagglutinin 1) coupled to keyhole limpet hemocyanin (KLH).
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 HA-epitope-tagged fusion proteins.
4. Hybridomas secreting monoclonal antibodies specific for the HA-epitope were isolated and cloned by limiting dilution.
5. The antibody was purified from bioreactor supernatants and lyophilized in the presence of proteinous stabilizers.

3.2. Quality Control

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

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		
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
Streptavidin Conjugates	Streptavidin-AP Conjugate, 1,000 U	11 089 161 001
	Streptavidin-β-Gal Conjugate, 500 U, *Not available in US*	11 112 481 001
	Streptavidin-POD Conjugate, 500 U	11 089 153 001
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
Tris hydrochloride	500 g	10 812 846 001
Tris base	1 kg, *Not available in US*	10 708 976 001
	1 kg	03 118 142 001
	5 kg	11 814 273 001
Nonidet P-40 Substitute	100 ml	11 754 599 001
cOmplete	20 tablets in a glass vial, for 50 ml each	11 697 498 001
	3 x 20 tablets in glass vials, for 50 ml each	11 836 145 001
cOmplete, Mini	25 tablets in a glass vial, for 10 ml each	11 836 153 001

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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