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Not for use in diagnostic procedures.

Anti-HA-Biotin, High Affinity
from rat IgG_1 (clone BMG 3F10)

Version: 07
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Monoclonal antibody for the highly sensitive detection of HA-tagged recombinant proteins, F_{ab} fragments conjugated with biotin.

Cat. No. 12 158 167 001 50 µg

Store the lyophilizate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Anti-HA-Biotin, High Affinity (3F10)	▪ White lyophilizate ▪ Lyophilized in the presence of proteinous stabilizers.	1 vial, 50 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-Biotin, High Affinity (3F10)	Store at +2 to +8°C.

Reconstitution

1. Add 1 ml double-distilled water to the lyophilizate to a final concentration of 50 µg/ml. Let stand 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.***

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*
- PVDF Western Blotting Membranes*
- Tween 20*
- BM Chemiluminescence Blotting Substrate (POD)*
- Streptavidin-Peroxidase*
- Blocking Reagent*
- Lumi-Film Chemiluminescent Detection Film*
- Na_2HPO_4, analysis grade
- NaH_2PO_4, analysis grade

For ELISA

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

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

1.4. Application

Anti-HA-Biotin, 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 and western blots.
- 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-HA-Biotin 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-HA-Biotin working solution	Dilute the reconstituted antibody to 100 ng/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-HA-Biotin 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-HA-Biotin working solution	Dilute the reconstituted antibody to 100 ng/ml using Blocking solution.	Unstable, prepare immediately before use.	Detection

2.2. Protocols

Sample preparation

Prepare protein extracts containing the HA-tagged protein of interest using a variety of standard methods.
The following lysis buffers have performed well:
- Bacterial extracts: 20 mM Tris, pH 8.0; 100 mM NaCl, Complete Protease Inhibitor*. (followed by sonication/ freeze-thaw).
- Mammalian extracts: 50 mM Tris, pH 7.5; 150 mM NaCl, 1% Nonidet P-40*, 0.05% deoxycholate, Complete Protease Inhibitor*.

Other cell lysis buffers may be more appropriate for individual applications.
- Include protease inhibitors to reduce proteolytic activity. Use Complete tablets* for most applications.
- Limit detergents to the lowest concentration levels necessary to obtain adequate cell lysis.

Western blotting

The following procedure describes the detection of a HA-tagged protein by enzyme-mediated chemiluminescence. If using other detection systems, such as colorimetric, the conditions may need to be adapted.
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 +37°C or for 3 hours at +15 to +25°C.
3. Incubate the blot with 100 ng/ml Anti-HA-Biotin solution for 1 hour at +15 to +25°C.
4. Wash 3 × 5 minutes with Washing solution.
5. Incubate the blot with 15 mU/ml Anti-Biotin-Peroxidase or 15 mU/ml Streptavidin-Peroxidase* diluted in Blocking solution for 1 hour at +15 to +25°C.

6 Wash 3 × 5 minutes with Washing solution.

7 Detect bound immuncomplexes with a chemiluminescence substrate as described in the Instructions for Use of the BM Chemiluminescence Blotting Substrate (POD)*.

Figure 1 shows a typical result regarding the specificity and sensitivity of the detection of HA-tagged proteins by western blotting.

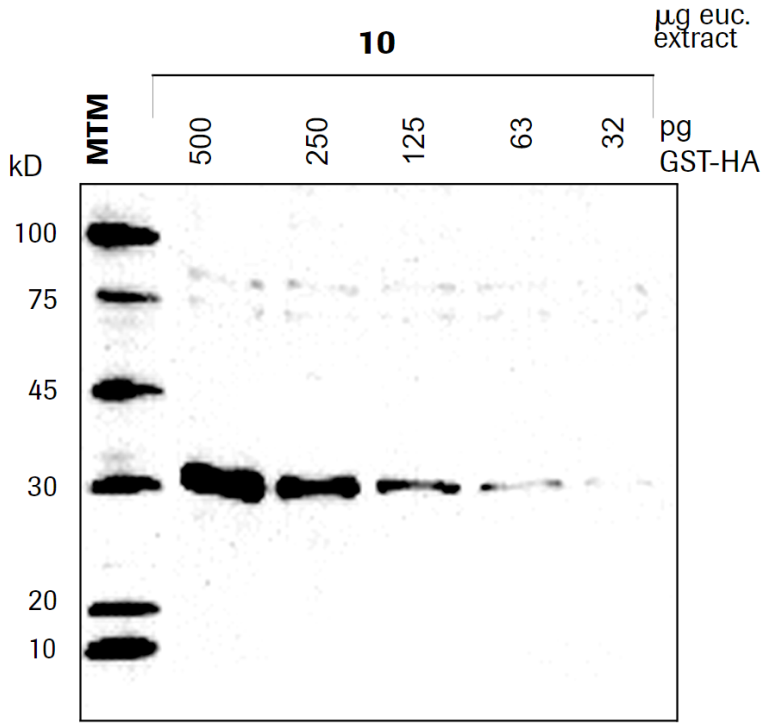

Fig. 1: Western blot analysis of HA-tagged Glutathione-S-transferase (GST-HA) detected with Anti-HA-Biotin, High Affinity(3F10).
Purified GST-HA was serially diluted to the indicated amounts in 10 µg of protein from eukaryotic cell extract. HA-tagged proteins were detected with 100 ng/ml Anti-HA-Biotin, High affinity (3F10), 20 mU/ml Anti-Biotin-Peroxidase, and BM Chemiluminescence Blotting substrate (POD), used according to the substrate's Instructions for Use (3 minute exposure). The observed background activity is derived from nonspecific binding of the secondary detection antibody (data not shown). MTM: Multi-Tag-Marker.

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 the 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 times 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 times with Washing solution, removing residual solution.

5 Add 100 µl Anti-HA-Biotin solution per well.
– Incubate for 1 hour at +15 to +25°C.

6 Wash 5 times with Washing solution, removing residual solution.

7 Add 100 µl Anti-Biotin-Peroxidase (15 mU/ml in Blocking solution) per well.
– Incubate for 10 minutes at +15 to +25°C.

8 Wash 5 times with Washing solution, removing residual solution.

9 Add 100 µl/well BM Blue POD Substrate, soluble*, prewarmed to +15 to +25°C.
– Incubate at +15 to +25°C and under constant shaking until the color development is sufficient.

10 To stop the color development, add 100 µl/well 2 M sulfuric acid.

11 Read the absorbance at 450 nm (reference wavelength: 690 nm) within 30 minutes after stopping the reaction.

2.3. Parameters

Specificity

Anti-HA-Biotin, 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 10 µg/ml
- Western and dot blot: 100 ng/ml

3. Troubleshooting

Observation	Possible cause	Recommendation
Nonspecific reactivity, especially with high total protein loading.	Nonspecific binding of secondary antibody.	Optimize assay conditions by reducing the concentration of the secondary antibody.
	Inadequate buffer conditions.	Prolong time for blocking the membrane.
		Reduce amount of total protein loaded.
		Use PBS containing Blocking reagent for membrane blocking, dilution of the Anti-HA-Biotin, and dilution of the secondary detection antibody.
	High Anti-HA-Biotin antibody concentration.	Reduce Anti-HA-Biotin antibody concentration.
Staining of the protein of interest is too weak.	Inadequate amounts of protein loaded onto the gel.	Increase the amount of total protein loading.
	Inadequate conditions used for detection.	Increase the concentration of Anti-HA-Biotin.
		Prolong exposure time used during detection.
Staining of the protein of interest is too strong.	Inadequate amounts of protein loaded onto the gel.	Decrease the amount of total protein loading.
	Inadequate conditions used for detection.	Decrease the concentration of Anti-HA-Biotin.
		Decrease the concentration of the secondary detection reagent.
		Shorten exposure time used during detection.

4. Additional Information on this Product

4.1. 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.

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
Lumi-Film Chemiluminescent Detection Film	100 films, 8 x 10 inches, 20.3 x 25.4 cm	11 666 657 001
cOmplete, EDTA-free	20 tablets in a glass vial, for 50 ml each	11 873 580 001
	3 x 20 tablets in glass vials, for 50 ml each	05 056 489 001
cOmplete, Mini, EDTA-free	25 tablets in a glass vial, for 10 ml each	11 836 170 001
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

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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