Anti-HA-Biotin, High Affinity (3F10)

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

Version 06
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Store at +2 to +8°C

1. Product characteristics

Antibody type

Clone BMG-3F10, rat IgG_1, F_{ab} fragments

Specificity

Anti-HA-Biotin, High Affinity (3F10) recognizes the HA peptide sequence [YPYDVPDYA] derived from the human influenza hemagglutinin protein (1). 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".

Formulation

White lyophilizate, lyophilized in the presence of proteinous stabilizers.

Storage and stability

The lyophilized Anti-HA-Biotin, High Affinity (3F10) is stable for 24 months or through the expiration date printed on the label when stored at 2−8°C. After reconstitution, the conjugate is stable for 2 month at +2 to +8°C. Alternatively, it can be stored in aliquots at −15 to −25°C. The reconstituted conjugate is stable for 6 month at −15 to −25°C. Repeated freezing and thawing must be avoided.

The Anti-HA-Biotin, High Affinity (3F10) is shipped at RT.

Reconstitution and storage

- Reconstitute the lyophilizate in 1.0 ml double distilled water for 10 minutes at 15 to 25°C (RT), and mix thoroughly but do not vortex.
- This results in a final concentration of 50 µg/ml.
- The reconstituted antibody is stable for
  – 2 months when stored at 2−8°C,
  or for– 6 months when stored in aliquots at −15 to −25°C. Repeated freeze/thaw cycles must be avoided !

Application

The following table lists the possible applications and recommended working concentrations:

Application	Working concentration
Western blot, Dot blot	100 ng/ml
ELISA	100 ng/ml

Quality control

The Anti-HA-Biotin, High Affinity (3F10) antibody is function tested by Western blot analysis of an HA-tagged fusion protein.

Advantages

Benefits	Features
Sensitivity	Anti-HA-Biotin, High Affinity (3F10) provides superior detection of HA-tagged proteins in the picogram range.
Specificity	No cross reactivity compared to other Anti-HA antibodies.
Flexibility	The biotin conjugated antibody allows assay establishing using the universal biotin-streptavidin platform.

2. Background information

Epitope tagging

Epitope tagging, the fusion of a short stretch of amino acids to a protein of interest by recombinant techniques, is a widely used method that allows the surveillance of the fusion protein with tag-specific monoclonal antibodies. The epitope tagging approach offers the ability 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 cells
- 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 and
- eliminates need to generate specific antibodies recognizing the protein of interest (1-6).

Anti-HA antibodies in epitope tagging

Among the different epitope-tags described in the literature the most commonly used tag is derived from the hemagglutinin of the influenza virus (HA1; 2). Several antibodies have been described that react with this epitope tag, the most prominent one is Anti-HA (2; 12CA5). However these antibodies are restricted by requiring additional amino acids adjacent to the HA tag or by recognizing HA-tagged proteins with only moderate affinity, as demonstrated by cross-reacting bands that have been reported in certain Western blot experiments (7).

Anti-HA, High Affinity

The Anti-HA High Affinity antibody (clone 3F10) recognizes the same epitope as clone 12CA5. It is a monoclonal antibody whose high affinity and low working concentrations result in less cross reactivity than with other antibodies to the HA-epitope. Anti-HA-Biotin, High Affinity (3F10) is a biotin conjugate of this clone which is especially useful in Western blotting, ELISA applications and assays using the universal biotin-streptavidin platform by allowing specific and highly sensitive detection of HA-tagged proteins.

3. Applications

3.1 Procedure for Western blotting

Introduction

The following procedure describes the detection of a HA-tagged protein by enzyme-mediated chemiluminescence.

If using other detection systems *e.g.*, colorimetric detection, the conditions may vary and should be adapted.

Before you begin

For separation by gel electrophoresis and blotting, please refer to reference 8.

Additional material required

The following table lists additional products from Roche Diagnostics necessary to perform the Western blotting procedure.

Product	Cat. No.
PVDF Western Blotting Membranes	11 722 034 001 11 722 026 001
Tween 20	11 332 465 001
BM Chemiluminescence Blotting Substrate (POD)	11 500 708 001 11 500 694 001
Blocking Reagent	11 096 176 001
Lumi-Film Chemiluminescent Detection Film	11 666 916 001 11 666 657 001 11 666 711 001
Na_2HPO_4, analysis grade	
NaH_2PO_4, analysis grade	

Preparation of working solutions

Working solution	Composition or preparation	Storage and stability	Use
Phosphate buffered saline (PBS), 10 ×	100 mM phosphate, 1.5 M NaCl, pH 7.2	stable for • 1 week at +2 to +8°C, or • at least 2 years at −15 to −25°C	Preparation of 1 × PBS
PBS, 1 ×	Dilute 10 ml 10× PBS with double dist. water to make 100 ml	stable for • 1 week at+2 to +8°C, or • at least 2 years at −15 to −25°C	• Preparation of blocking solution • Preparation of Washing solution
Washing solution	PBS, 1 ×, containing 0.1% Tween 20 (v/v)	stable for 1 week at +2 to +8°C	Washing
Blocking solution	PBS, 1×, containing 1% Blocking Reagent (w/v)	stable for • 1 week at +2 to +8°C, or • at least 2 years at −15 to −25°C	• Blocking • Preparation of Anti-HA-Biotin Working solution
Anti-HA-Biotin solution	Dilute the reconstituted antibody to 100 ng/ml using the Blocking solution	unstable, prepare shortly before use	Detection

Important notes regarding sample preparation

Prepare protein extracts containing the HA tagged protein of interest using a variety of standard methods (8). The following lysis buffers have performed well:

- Bacterial extracts: 20 mM Tris, pH 8.0; 100 mM NaCl, Complete[1)] Protease Inhibitor. (followed by sonication/ freeze-thaw).
- Mammalian extracts: 50 mM Tris, pH 7.5; 150 mM NaCl, 1% Nonidet 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.

Procedure for immunodetection

Step	Action
1	After electrophoresis and transfer of the proteins to a PVDF membrane, block the membrane with blocking solution for 1 h at +37 °C or for 3 h at +15 to +25°C.
2	Incubate the blot with 100 ng/ml Anti-HA-Biotin working solution for 1 h at +15 to +25°C.
3	Wash 3×, 5 min each, with washing solution.
4	Incubate the blot with 15 mU/ml Anti-Biotin-Peroxidase* or 15 mU/ml Streptavidin-Peroxidase* diluted in Blocking solution for 1 h at +15 to +25°C.
5	Wash 3×, 5 min each, with washing solution.
6	Detect bound immuncomplexes with a chemiluminescence substrate as described in the package insert of the BM Chemiluminescence Blotting Substrate (POD).

Typical result

The following picture shows a typical result regarding the specificity and sensitivity of the detection of HA-tagged proteins by Western blotting.

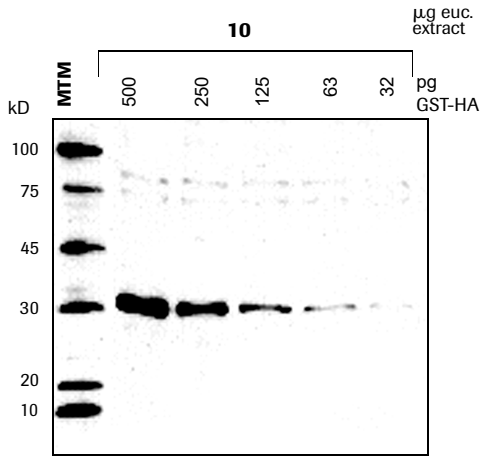

Fig. 1: Western blot analysis of HA-tagged Glutathion-S-transferase (GST-HA) detected with Anti-HA-Biotin, High Affinity (3F10).

Purified GST-HA was serially diluted to the indicated amounts in 10 µg of protein from eucaryotic 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 package insert (3 min exposure). The observed background activity is derived from non-specific binding of the secondary detection antibody (data not shown).

MTM: Multi-Tag-Marker

3.2 Procedure for ELISA

Before you begin

For detailed information, please refer to reference No. 8.

Additional equipment required

- Microtiter plates (*e.g.*, Nunc Maxisorp)
- Microtiter plate washer (optional)
- Microtiter plate reader

Additional reagents required

The following table lists additional products required for the ELISA procedure

Product	Cat. No.
Tween 20	11 332 465 001
Blocking Reagent	11 096 176 001
BM Blue POD Substrate, soluble	11 484 281 001
Sodium carbonate, analysis grade	
Sulfuric acid, 95–97%, analysis grade	

Preparation of working solutions

Working solution	Composition or preparation	Storage and stability	Use
Sodium carbonate solution	50 mM, pH 9.6	prepare shortly before use	Coating
Phosphate buffered saline (PBS), 10 ×	100 mM phosphate, 1.5 M NaCl, pH 7.2	stable for • 1 week at 2−8°C, or • at least 2 years at −15 to −25°C	Preparation of 1× PBS
PBS, 1×	Dilute 10 ml 10 × PBS with double dist. water to make 100 ml	stable for • 1 week at 2−8°C, or • at least 2 years at −15 to −25°C	• Preparation of blocking solution • Preparation of Washing solution
Washing solution	1× PBS containing 0.1 % Tween 20 (v/v)	stable for 1 week at 2−8°C	Washing
Blocking solution	1× PBS containing 1% Blocking Reagent (w/v)	stable for • 1 week at 2−8°C, or • at least 2 years at −15 to −25°C	• Blocking • Preparation of Anti-HA-Biotin Working solution
Coating solution	Dilute 1–10 µg of the appropriate protein in 1 ml sodium carbonate solution	prepare shortly before use	Coating
Anti-HA-Biotin working solution	Dilute the reconstituted antibody to 100 ng/ml using the Blocking solution	unstable; prepare shortly before use	Detection

Procedure for ELISA

Cover the plate either with microtiter plate covers or adhesive sealing film during all incubation steps in order to avoid evaporation of the solutions.

Step	Action
1	Coat the wells with 100 µl/well coating solution for 1–2 h at 37°C or over night at +2 to +8°C.
2	Wash 5 × with washing solution and remove residual washing solution.
3	Add 300 µl blocking solution per well and incubate for 1–2 h at 37 °C or over night at +2 to +8°C.
4	Wash 5 × with washing solution and remove residual washing solution.
5	Add 100 µl Anti-HA-Biotin working solution per well, and incubate for 1 h at +15 to +25°C.
6	Wash 5 × with washing solution and remove residual washing solution.
7	Add 100 µl Anti-Biotin-Peroxidase (15 mU/ml in blocking solution) per well, and incubate for 10 min at +15 to +25°C.
8	Wash 5× with washing solution and remove residual washing solution.
9	Add 100 µl/well RMB Blue POD Substrate, soluble, prewarmed to +15 to +25°C, and 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 N sulphuric acid.
11	Read the absorbance at 450 nm (reference wavelength: 690 nm) within 30 min after stopping the reaction.

4. Appendix

4.1 Trouble shooting

Problem	Possible Cause	Recommendation
Nonspecific reactivity especially with high total protein loading.	• Nonspecific binding of secondary antibody. • Inadequate buffer conditions. • High Anti-HA-Biotin antibody concentration	• Optimise assay conditions by reducing the concentration of the secondary antibody. • 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. • Reduce Anti-HA-Biotin antibody concentration
Staining of the protein of interest is too weak.	• Inadequate amounts of protein loaded onto the gel. • Inadequate conditions used for detection.	• Increase the amount total protein loading. • 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. • Inadequate conditions used for detection	• Decrease the amount total protein loading. • Decrease the concentration of Anti-HA-Biotin. • Decrease the concentration of the secondary detection reagent. • Shorten exposure time used during detection.

4.2 References

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* *available from Roche Diagnostics*

Changes to previous version Editorial changes.

4.3 Related products

Product	Pack size	Cat. No.
Anti-HA, High Affinity (3F10)		
lyophilized, unconjugated	50 µg	11 867 423 001
	500 µg	11 867 431 001
peroxidase	25 U (25 µg)	12 013 819 001
HA peptide	5 mg	11 666 975 001
Anti-HA (12CA5)		
lyophilized, unconjugated	200 µg	11 583 816 001
in solution, unconjugated	5 mg	11 666 606 001
biotin	100 µg (500 µl)	11 666 851 001
fluorescein	100 µg (500 µl)	11 666 878 001
rhodamine	100 µg (500 µl)	11 666 959 001
peroxidase	50 µg (500 µl)	11 667 475 001
Anti-Biotin-Peroxidase	150 units	11 426 303 001
Streptavidin-Peroxidase	500 units	11 089 153 001
Anti-c-myc		
lyophilized, unconjugated	200 µg	11 667 149 001
in solution, unconjugated	5 mg	11 667 203 001
peroxidase	500 µg (500 µl)	11 814 150 001
Anti-VSV-G		
lyophilized, unconjugated	200 µg	11 667 351 001
Anti-His_6	100 µg	11 922 416 001
Anti-His_6-Peroxidase	50 units	11 965 085 001
Anti-GFP	200 µg	11 814 460 001
rGFP	50 µg	11 814 524 001
c-myc peptide	5 mg	11 667 246 001
Multi-Tag Marker	250 µl	11 828 649 001
X-tremeGENE siRNA Transfection Reagent	1 ml, 400 Transfections 5× 1 ml, 2000 transfections	04 476 093 001 04 476 115 001
FuGENE 6 Transfection Reagent	1 ml	11 814 443 001
DOSPER	2 ml (5 × 0.4 ml)	11 781 995 001
Liposomal Transfection Reagent	0.4 ml	11 811 169 001
DOTAP	2 ml (5 × 0.4 ml)	11 202 375 001
Liposomal Transfection Reagent	0.4 ml	11 811 177 001
Lumi Light^{PLUS} POD Western Blotting Kit	1000 cm^2	12 015 218 001
Lumi Light^{PLUS} POD Western Blotting Substrate	100 ml	12 015 196 001
Lumi Light POD Western Blotting Substrate	400 ml	12 015 200 001
BM Blue POD Substrate, precipitating	100 ml	11 442 066 001
Lumi-Film Chemiluminescent Detection Film	100 films (8 × 10 inches 20.3 × 25.4 cm)	11 666 657 001
Complete, EDTA-free Protease Inhibitor Cocktail Tablets	20 tablets (each sufficient for 50 ml extract)	11 873 580 001
Complete, Mini, EDTA-free Protease Inhibitor Cocktail Tablets	25 tablets (each sufficient for 10 ml extract)	11 836 170 001

Contact and Support

To ask questions, solve problems, suggest enhancements and 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.

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany