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FOR *IN VITRO* USE ONLY.

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

Rat monoclonal antibody for the highly sensitive detection of HA-tagged recombinant proteins, conjugated with peroxidase

Cat. No. 12 013 819 001

25 U (25 µg)

Version June 2005

Store at +2 to +8°C

1. Product characteristics

Antibody type

Clone BMG-3F10, rat IgG_1

Specificity

Anti-HA-Peroxidase, High Affinity (3F10) recognizes the HA peptide sequence [YPYDVPDYA] derived from the 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-Peroxidase, High Affinity (3F10) is stable for 24 months or through the expiration date printed on the label when stored at +2 to +8°C. The Anti-HA-Peroxidase, High Affinity (3F10) is shipped at +15 to +25°C.

Reconstitution and storage

- Reconstitute the lyophilizate in 1.0 ml double distilled water for 10 minutes at +15 to +25°C, and mix thoroughly but do not vortex.
- This results in a final concentration of 25 U/ml.
- The reconstituted antibody is stable for
  - 2 months when stored at +2 to +8°C, *or for*
  - 6 months when stored in aliquots at −15 to −25°C. Repeated freeze/thaw cycles must be avoided because that may affect the peroxidase activity!

Note: DO NOT add sodium azide as a preservative because it inhibits the activity of the peroxidase!

Application

Anti-HA-peroxidase is used for single-step detection of HA-tagged recombinant proteins by Western blot analysis.

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

Application	Working concentration
Western blot, Dot blot	50 mU/ml
ELISA	25 mU/ml

Advantages

Benefits	Features
Sensitivity	Anti-HA-Peroxidase, High Affinity (3F10) provides superior detection of HA-tagged proteins in the picogram range.
Specificity	No cross reactivity compared to other Anti-HA antibodies.
Convenience	The peroxidase conjugated antibody reduces the total assay time and number of handling steps because no secondary detection antibody is necessary in immunoblots and ELISA procedures.
Clear results	Using samples obtained by immunoprecipitation, there is no interference with the precipitating antibody in immunoblots.

Quality control

The Anti-HA-Peroxidase, High Affinity (3F10) antibody is function tested by Western blot analysis using a cell line, that expresses a recombinant HA-tagged protein.

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 one is HA. It is derived from the hemagglutinin of the influenza virus (2). Several antibodies have been described that react with this epitope tag, the most prominent one is Anti-HA (2; clone 12CA5). However these antibodies are restricted by requiring additional amino acids adjacent to the HA tag or recognize HA-tagged proteins with only moderate affinity e.g. as demonstrated by cross-reacting bands that have been reported in certain Western blot experiments using Anti-HA (clone 12CA5) (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 concentration result in less cross reactivity when compared with other antibodies to the HA-epitope. Anti-HA-Peroxidase, High Affinity (3F10) is a horseradish peroxidase conjugate of this clone which is especially useful in Western blot and ELISA applications by allowing specific and highly sensitive detection of HA-tagged proteins.

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3. Protocols

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 have to be adapted.

Before you begin

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

Additional material required

- PVDF Western Blotting Membranes*
- Tween 20*
- BM Chemiluminescence Blotting Substrate (POD)*
- Blocking Reagent *
- Lumi-Film Chemiluminescent Detection Film*
- 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 2-8°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 • Washing solution • Anti-HA-Peroxidase solution
Washing solution	PBS, 1×, containing 0.1% Tween 20 (v/v)	stable for 1 week at 2-8°C	Washing
Blocking solution	PBS, 1×, containing 1% (w/v) Blocking Reagent	stable for • 1 week at 2-8°C, or • at least 2 years at −15 to −25°C	Blocking
Anti-HA-Peroxidase solution	Dilute the reconstituted antibody to 50 mU/ml using the Blocking solution	unstable, prepare shortly before use	Detection

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 50 mU/ml Anti-HA-Peroxidase solution for 1 h at +15 to +25°C
3	Wash 4× 10 min each, with Washing solution.
4	Detect bound immunocomplexes 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.

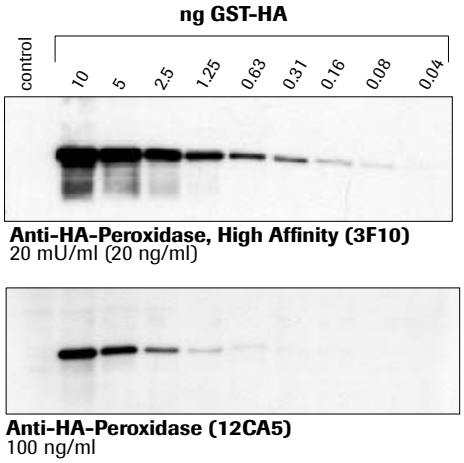

Fig. 1: Western blot analysis of HA-tagged Glutathion-S-transferase (GST-HA) detected with Anti-HA-Peroxidase, High Affinity (3F10) and Anti-HA-Peroxidase (12CA5). Purified GST-HA was serially diluted to the indicated amounts in 15 µg of eucaryotic protein background and detected with the indicated amounts of Anti-HA-Peroxidase, High Affinity (3F10) and Anti-HA-Peroxidase (12CA5) according to the package inserts using the BM Chemiluminescence Blotting substrate (POD) (3 min exposures). The control lane is an untransfected eucaryotic cell extract (15 µg total protein).

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3.2 Procedure for ELISA

Before you begin

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

Additional required equipment

- Microplates (*e.g.*, Nunc Maxisorp)
- Microplate washer (optional)
- Microplate reader

Additional required reagents

- Tween 20*
- Blocking Reagent*
- BM Blue POD Substrate, soluble*
- 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 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 • Washing solution • Anti-HA-Peroxidase solution
Washing solution	PBS, 1×, containing 0.1 % Tween 20 (v/v)	stable for 1 week at 2-8°C	Washing
Blocking solution	PBS, 1×, containing 1% (w/v) Blocking Reagent	stable for • 1 week at +2 to +8°C, or • at least 2 years at −15 to −25°C	Blocking
Coating solution	Dilute 1–10 µg of the appropriate protein in 1 ml sodium carbonate solution	prepare shortly before use	Coating
Anti-HA-Peroxidase solution	Dilute the reconstituted antibody to 25 mU/ml using the Blocking solution	unstable; prepare shortly before use	Detection

Procedure for ELISA

Cover the plate either with adhesive cover foils or special microtiter plates 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 overnight 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 overnight at +2 to +8°C.
4	Wash 5× with Washing solution and remove residual washing solution.
5	Add 100 µl Anti-HA-Peroxidase 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/well BM 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.
8	Add 100 µl/well 2 N sulfuric acid to stop the color development.
9	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	• Inadequate buffer conditions	• Use PBS containing Blocking reagent for membrane blocking and dilution of the Anti-HA-Peroxidase. • Prolong time for blocking the membrane.
	• Inadequate washing	• Increase washing time.
Staining of the protein of interest is too weak or too strong.	• Inadequate amounts of protein loaded onto the gel • Inadequate conditions used for detection	• Increase or decrease the amount total protein loading. • Increase or decrease the concentration of Anti-HA-Peroxidase. • Shorten or prolong exposure time used during detection.

4.2 References

1 Kolodziej, P.A. & Young, R.A. Epitope Tagging and Protein Surveillance.
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3 Field, J., Nikawa, J.-I., Broek, D., MacDonald, B., Rodgers, L., Wilson, I.A., Lerner, R.A. & Wigler, M. (1988): Purification of a RAS-Responsive Adenylyl Cyclase Complex from Saccharomyces cerevisiae by Use of an Epitope Addition Method.
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Biochem. Biophys. Res. Com. **197**, 214-220.

6 Qian, N.-X., Winitz, S. & Johnson, G.L. (1993): Epitope-tagged Gq a subunits: Expression of GTPase-deficient a subunits persistently stimulates phosphatidylinositol-specific phospholipase C but not mitogen-activated protein kinase activity regulated by the M1 muscarinergic acetylcholin receptor. *Proc. Natl. Acad. Sci.* **90**, 4077-4081.

7 Chen, Y.-T., Holcomb, C. & Moore, H.-P.H (1993): Expression and localization of two low molecular weight GTP-binding proteins, Rab8 and Rab19, by epitope tag.
Proc. Natl. Acad. Sci. **90**, 6508-6512.

8 Harlow, E. & Lane, D. (1988): Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, N.Y.

4.3 Ordering Information

Product	Pack size	Cat. No.
Anti-HA, High Affinity (3F10)	50 µg 500 µg	11 867 423 001 11 867 431 001
Anti-HA (12CA5)	200 µg	11 583 816 001
Anti-HA-Biotin (12CA5)	100 µg	11 666 851 001
Anti-HA-Biotin High Affinity (3F10)	50 µg	12 158 167 001
Anti-HA-Rhodamine (12CA5)	100 µg (500 µl)	11 666 959 001
HA Peptide	5 mg	11 666 975 001
c-myc peptide	5 mg	11 667 246 001
Anti-VSV-G (P5D4)	200 µg	11 667 351 001
Anti-HA Affinity Matrix (3F10)	1 ml	11 815 016 001
Anti-Protein C Affinity Matrix (HPC4)	1 ml	11 815 024 001
Anti-HA-Peroxidase (12CA5)	50 µg	11 667 475 001
Anti-HA-Peroxidase, High Affinity (3F10)	25 U (25 µg)	12 013 819 001
Multi-Tag-Marker	250 µl	11 828 649 001
Anti-His_6	100 µg	11 922 416 001
Anti-His_6-Peroxidase	50 U	11 965 085 001
Anti-c-myc	200 µg	11 667 149 001
Anti-c-myc-Peroxidase	500 µg	11 814 150 001
Anti-Protein C	200 µg	1 814 5081 001
Anti-Protein C-Peroxidase	50 µg	11 814 974 001
BM Blue POD Substrate, soluble	100 ml	11 484 281 001
Blocking Reagent	50 g	11 096 176 001
PVDF Western Blotting Membranes	1 roll (30 cm × 3 m)	03 010 040 001
Tween 20	5 × 10 ml	11 332 465 001
BM Chemiluminescence Western Blotting Substrate (POD)	1 set (1,000 cm^2 membrane) 1 set (4,000 cm^2 membrane)	11 500 708 001 11 500 694 001
Lumi-Film Chemiluminescent Detection Film	100 films (8 × 10 inches))	11 666 657 001

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Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany