For life science research only. Not for use in
diagnostic procedures. FOR *IN VITRO* USE ONLY.

Anti-HA (12CA5)

Mouse monoclonal antibody (clone 12CA5) to a peptide epitope derived from the hemagglutinin protein of human influenza virus

Cat. No. 11 583 816 001 200 µg (lyophilized)
Cat. No. 11 666 606 001 5 mg (1 ml solution)

Version July 2009

Store at −15 to −25°C

1. What this Product Does

Contents

- Cat. No. 11 583 816 001: 200 µg white lyophilizate
- Cat. No. 11 666 606 001: 1 ml frozen liquid (5 mg/ml)

Storage and Stability

If stored at −15 to −25°C, the lyophilized antibody preparations are stable through the expiration date printed on the label.

For storage, prepare convenient aliquots and freeze them at −15 to −25°C.

⚠ Avoid repeated freezing and thawing.

Lyophilized Anti-HA antibody is shipped at ambient temperature. Liquid Anti-HA antibody is shipped on dry ice.

Application

Anti-HA (12CA5) is used for the immunochemical detection of native influenza hemagglutinin protein and recombinant "epitope tagged" proteins containing the HA epitope in western and dot blots, immunocytochemistry, and immunoprecipitation.

For experiments in which sensitivity is not critical, use Anti-HA (12CA5). For higher sensitivity detection in western blotting at 10-fold lower concentration use Anti-HA , High Affinity (3F10)*.

Additional Equipment and Reagents Required

Additional equipment and reagents required to perform western blot analyses using Anti-HA (12CA5) include:

- Western blotting apparatus
- Western Transfer Buffer: 10% methanol, 24 mM Tris-base, 194 mM glycine
- PVDF Western Blotting Membrane*
- Washing Buffer (PBST): PBS, 0.05–2% Tween 20, pH 7.5
- Blocking Solution (1:10 dilution of Western Blocking Reagent* in 1× PBST
- secondary, POD-conjugated anti-mouse antibody (*e.g.,* Goat Anti-Mouse (H+L)-POD)
- Lumi-Light Western Blotting Substrate* or Lumi-Light^{PLUS} Western Blotting Substrate*
- Lumi-Film Chemiluminescent Detection Film*

Product Characteristics

Specificity	Anti-HA recognizes the HA nonapeptide sequence YPYDVPDYA derived from the human influenza virus hemagglutinin protein (amino acids 98-106) (1). The antibody recognizes its antigenic determinant even when the HA peptide epitope is introduced into unrelated recombinant proteins using a technique known as "epitope tagging."
Clone	12CA5 (2)
Subtype	Mouse $IgG_{2b}\kappa$
Purity	The anti-HA monoclonal antibody is ≥90% pure as determined by HPLC. The antibody preparation does not contain preservatives or stabilizers.
Affinity	$K_a = 1 \times 10^8$/M
Isoelectric Point	6.4

2. How to Use this Product

2.1 Before You Begin

Epitope Tagging

Before using Anti-HA to analyze the product of your target gene, incorporate the 27-base DNA sequence, which encodes the HA epitope, into the target gene sequence using one of the following methods:

- Clone your gene of interest into a suitable bacterial or mammalian expression vector.
- Prepare oligonucleotide linkers that can encode the HA epitope, and clone the linkers into the target gene at the desired N-terminal, C-terminal, or internal site (3).
- Insert the HA-peptide coding sequence into the target gene by oligonucleotide-mediated site-directed mutagenesis (4,5).

Preparation of Working Solutions

For the lyophilized anti-HA preparation, prepare a concentrated stock solution (0.4 mg/ml) by dissolving the entire lyophilized anti-HA antibody (200 µg) in 500 µl PBS (rehydrate for 30 min prior to use). For storage, prepare convenient aliquots and freeze them at –15 to –25°C.

The liquid anti-HA preparation (5 mg/ml) is ready to use as supplied.

For most applications, dilute the Anti-HA antibody concentrate in an appropriate buffer to a concentration range of 0.1–5 µg/ml. Determine optimal dilution buffer and dilution conditions for each specific application and method.

2.2 Procedure

Western Blotting Analysis

To perform western blotting analysis using the anti-HA (12CA5), follow the protocol below:

Step	Action
❶	Perform western blot transfer to a PVDF membrane* (0.45-µm pore size) using Western Transfer Buffer. ⚠ The buffer must be at +2 to +8°C for electrophoretic transfer.
❷	Transfer the membrane (protein side up) to a container large enough to hold it. ⚠ Be sure the side of the membrane that contains protein faces up so the detection reagents will have maximum access to the antigens. 🔍 A disposable, square petri dish (100 × 15 mm) makes a convenient container for a 10 × 10 cm blot.
❸	• After transfer, block the membrane for 1 hour at +15 to +25°C with 10 ml#) of Blocking Solution • Place container on a rotating platform and rotate gently for 1 h at +15 to +25°C. ⚠ Make sure the reagent completely and constantly covers the membrane during this incubation and all incubation steps below. 🔍 This incubation step may also be performed at +2 to +8°C O/N.
❹	Drain the Blocking Solution from the container and wash the blocked membrane once with PBST.
❺	Dilute anti-HA antibody to a concentration of 0.1–1.0 µg/ml in a 1:20 dilution of Western Blocking Reagent in PBST. Incubate membrane with diluted anti-HA antibody for 1 hour at +15 to +25°C with gentle rotation.
❻	• Drain the antibody solution from the container and rinse the membrane with approximately 20 ml#) of Wash Buffer. • Wash the membrane four times, 10 min per wash, with 20 ml#) PBST.
❼	Dilute secondary, POD-conjugated antibody 1:10,000 in diluent (as in step 5 above) and incubate the membrane for 1 h at +15 to +25°C with gentle rotation.
❽	Wash the membrane four times, 10 min per wash, with PBST. Prepare Lumi-Light Western Blotting Substrate* according to package insert instructions.
❾	• Drain the antibody solution from the container and rinse the membrane with approximately 20 ml#) of Wash Buffer. • Wash the membrane four times, 10 min per wash, with 20 ml#) PBST.
❿	Add the Lumi-Light reagent to the membrane.
⓫	Expose the membrane to X-ray film. For a 1 min substrate development, perform a 1–5 min exposure initially. 🔍 The conditions for development and exposure may vary.

#) volume required per 100 cm^2 membrane

Immunoprecipitation and immunoaffinity purification

Anti-HA antibody is commonly used to isolate HA-tagged proteins and associated protein:protein binding partners by immunoprecipitation (IP) or immunoaffinity purification. Numerous IP methods are found in the literature (2, 3, 5). Optimal conditions for IP should be determined for each particular application. Use the Anti-HA Affinity Matrix (HA clone 3F10)* for the direct immunoprecipitation or purification of HA-tagged proteins. The high-affinity HA antibody is covalently coupled to agarose beads, which enables the rapid isolation of even rarely expressed HA-tagged protein with minimal interference of heavy- and light-chain bands during western-blot analysis.

Immunofluorescence

Anti-HA antibody is commonly used to visualize HA-tagged fusion proteins in various cell types by indirect immunofluorescence (6, 8–17). For staining HA-tagged proteins in yeast, start with the method of Berkower et al. (17). For staining of HA-tagged proteins in mammalian cells, start with the method of Canfield and Levenson (11). Optimal conditions for staining and fluorescent microscopy should be determined for each particular application. The recommended starting concentration is 1 to 10 µg/ml.

🔍 For direct immunofluorescent detection, use anti-HA-Biotin, High Affinity (3F10) FITC*

🔍 Detailed working instructions for immunoaffinity purification and immunofluorescence detection can be found in *Epitope Tagging Basic Laboratory Methods* (https://www.roche-applied-science.com/PROD_INF/MANUALS/epitope/epi_toc.htm).

3. Troubleshooting

Problem	Possible Cause	Recommendation
Chemiluminescent or chromogenic signal weak or not visible	Poor isolation of tagged protein	Use a different cell lysis procedure
	Antibody too dilute	Double the concentration of the anti-HA and/or the secondary antibody)
	Too little protein on the gel	Add more protein to gel.
	Poor transfer of proteins from gel to membrane	• Increase the electrical current and/or the transfer time for the blot. • Be sure there are no air bubbles between the membrane and gel during transfer.
	Wrong type of membrane	For maximum signal, use PVDF membranes for transfer.
	Antibody incubation too short	Incubate anti-HA (and/or the secondary antibody) with the membrane blot for a longer time.
	Signal development time too short	Double the development time.
	Wash time too long or too stringent	• Shorten the washing time. • Omit Tween 20 from the Wash Buffer.
	Enzyme on antibody conjugate inactivated by preservative	Do not use sodium azide in any western blot reagent if you use POD-conjugated antibodies.
	Substrate inactive	Make fresh dilution of substrate or start with a different stock of substrate.
	Epitope tag sequence is not detectable due to: • Proteolytic cleavage • Low level of expression • Premature translation termination resulting in loss of C-terminal tag sequence	• Include protease inhibitors in lysis buffer. • Use alternative expression system or optimize your expression system. • Insert multiple tag sequences into target protein to increase avidity of antibody reaction. • Use alternative insertion site within the target gene for the epitope tag sequence.
High background, additional bands on blot	Antibody too concentrated	Decrease concentration of anti-HA (and/or Secondary Antibody) by half.
	Wash time too short	Wash time too short
	Incubation of membrane with substrate too long	Leave blot membrane in substrate for a shorter time.
	Wrong type membrane	For minimum background, use PVDF membranes for transfer.
	Blocking Reagent too dilute	Use nonfat dry milk (5% w/v) dissolved in Reagent Diluent as Blocking Reagent. ⚠ High concentrations of nonfat dry milk may reduce specific signal as well as background.)
	Contaminated reagents or equipment	• Use clean equipment, freshly prepared • buffers, and new membranes. • Always avoid touching membranes with bare hands; use gloves and forceps.

Problem	Possible Cause	Recommendation
	Secondary antibody binds untagged proteins.	Use an $F(ab')_2$ fragment of a secondary antibody, rather than an intact IgG.
	Heavy and light chains of primary antibody visible on blot membrane	Use direct detection with peroxidase-conjugated monoclonal antibody to visualize tagged proteins.
	Signal development time too long	Reduce development time by half.

4. Additional Information on this Product

Background Information

The anti-HA antibody was originally used (1) 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 DNA techniques (2, 3). The ability to prepare such epitope-tagged proteins and locate them with the anti-HA antibody in subsequent experiments (1, 2, 6–20) 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.

Preparation

Clone 12CA5 is a subclone of H26DO8. This parent clone was obtained by immunizing 129 GIX^+ mice with a synthetic peptide (residues 76–111 of X47 hemagglutinin 1) coupled to keyhole limpet hemocyanin. Spleen cells were then fused with SP2/0 myeloma cells in polyethylene glycol to create the H26DO8 hybridoma clone (21). Anti-HA antibody was purified and dialyzed against 10 mM potassium phosphate, 70 mM NaCl (pH 7.4), and either lyophilized (200 µg preparation) or adjusted to the correct concentration (5 mg/ml preparation).

Quality Control

Each lot of anti-HA antibody is tested for functionality and purity relative to a reference standard to confirm the quality of each new reagent preparation: a sample of a recombinant protein containing the HA epitope is resolved by SDS-PAGE and transferred to a PVDF membrane. When incubated with the blot membrane at a concentration of 0.1 µg antibody/ml, the anti-HA antibody binds specifically to the recombinant HA-tagged protein. The antigen/antibody complex on the membrane is visualized with an anti-[Mouse IgG]-HRP conjugate using a chemiluminescent substrate.

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Product Citations

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5. Supplementary Information

5.1 Conventions

Text Conventions

To make information consistent and easy-to-read, the following text conventions are used in this Instruction Manual:

Text Convention	Usage
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

Abbreviations

In this Instruction Manual the following abbreviations are used:

Abbreviation	Meaning
HA	hemagglutinin
PBS	phosphate-buffered saline
POD	peroxidase
PVDF	polyvinyl-difluoride
A; D ; P; V ; Y	alanine; aspartic acid; proline; valine; tyrosine

5.2 Changes to Previous Version

- Editorial changes
- Ordering Information revised

5.3 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com.

Further epitope tagging related products can be found under http://www.roche-applied-science.com/sis/proteomicscience/prot_characterization/epitope_tagging.htm.

Product	Pack Size	Cat No.
Anti-HA (12CA5)	200 µg 5 mg (1ml)	11 583 816 001 11 666 606 001
Anti-HA-Biotin	100 µg (500 µl)	11 666 851 001
Anti-HA-Fluorescein	100 µg (500 µl)	11 666 878 001
Anti-HA-Rhodamine	100 µg (500 µl)	11 666 959 001
Anti-HA High Affinity (3F10)	50 µg 500 µg	11 867 423 001 11 867 431 001
Anti-HA-Biotin, High Affinity (3F10)	50 µg	12 158 167 001
Anti-HA-Fluorescein, High Affinity (3F10)	25 µg	11 988 506 001
Anti-HA-Peroxidase, High Affinity (3F10)	25 µg	12 013 819 001
Anti-HA Affinity Matrix	1 ml	11 815 016 001
HA Peptide	5 mg	11 666 975 001
Protease/Phosphatase Inhibitor Tablets and Lysis Reagents		
c●mplete	20 tablets in glass vials 3 × 20 tablets in glass vials 20 tablets in *EASYpacks*	11 697 498 001 11 836 145 001 04 693 116 001
c●mplete, Mini	25 tablets in a glass vial 30 tablets in *EASYpacks*	11 836 153 001 04 693 124 001
c●mplete, EDTA-free	20 tablets in a glass vial 3 × 20 tablets in glass vials 20 tablets in *EASYpacks*	11 873 580 001 05 056 489 001 04 693 132 001
c●mplete, Mini, EDTA-free	25 tablets in a glass vial 30 tablets in *EASYpacks*	11 836 170 001 04 693 159 001
c●mplete Lysis-B (2×) (for bacterial cell lysis)	1 kit (100 ml lysis reagent and 20 c●mplete Protease Inhibitor Cocktail Tablets)	04 719 930 001
c●mplete Lysis-B (2×), EDTA-free (for bacterial cell lysis)	1 kit (100 ml lysis reagent and 20 c●mplete, EDTA-free Protease Inhibitor Cocktail Tablets)	04 719 948 001
c●mplete Lysis-M (for mammalian cell lysis)	1 kit (200 ml lysis reagent and 20 c●mplete Protease Inhibitor Cocktail Tablets)	04 719 956 001
c●mplete Lysis-M, EDTA-free (for mammalian cell lysis)	1 kit (200 ml lysis reagent and 20 c●mplete, EDTA-free Protease Inhibitor Cocktail Tablets)	04 719 964 001
PhosSTOP	20 tablets in *EASYpacks* 10 tablets in *EASYpacks*	04 906 837 001 04 906 845 001
Transfection Reagents		
FuGENE® 6 Transfection Reagent	0.4 ml (120 transfections) 1 ml (300 transfections) Multi-pack 5 × 1 ml (1,500 transfections)1 Mega-pack 5 × 1 ml (1,500 transfections) 10 ml (3,000 transfections)	11 815 091 001 11 814 443 001 11 815 075 001 11 988 387 001 05 061 377 001
FuGENE® HD Transfection Reagent	0.4 ml (120 transfections) 1 ml (300 transfections) Trial pack Mega-pack 5 × 1 ml (1,500 transfections) [1)] 10 ml (3,000 transfections) 1) The five vials are packaged together in one box with one pack insert.	04 709 691001 04 709 705 001 04 883 560 001 04 709 713 001 05 061 369 001
Western Blotting Reagents		
Lumi-Light^{PLUS} Western Blotting Kit (Mouse/Rabbit)	1 kit (1,000 cm^2 membrane)	12 015 218 001
Lumi-Light Western Blotting Substrate	400 ml, (4000 cm^2 membrane)	12 015 200 001
Lumi-Light^{PLUS} Western Blotting Substrate	100 ml, (1,000 cm^2 membrane)	12 015 196 001
Lumi-Film Chemiluminescent Detection Film	100 films (8 × 10 inches 20.3 x 25.4 cm)	11 666 657 001
PVDF Western Blotting Membranes	1 roll (30 cm × 3.00 m)	03 010 040 001
Western Blocking Reagent, Solution	100 ml (10 blots, 100 cm^2) 6 × 100 ml (60 blots, 100 cm^2)	11 921 673 001 11 921 681 001
Bovine Serum Albumin, Fraction V	50 g 100 g 500 g 1 kg	10 735 078 001 10 735 086 001 10 735 094 001 10 735 108 001
Detergents		
Triton X-100	5 × 10 ml	11 332 481 001
Tween 20	5 × 10 ml	11 332 465 001
Nonidet P40	5 × 10 ml	11 332 473 001
Buffers in a Box, Premixed PBS Buffer, 10×	4 l	11 666 789 001
Immunoprecipitation Reagents		
Immunoprecipitation Kit (Protein G)	20 reactions	11 719 386 001
Protein G Agarose	2 ml 5 ml 15 ml	11 719 416 001 11 243 233 001 05 015 952 001

#) *only available in the U.S.*

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