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

Anti-HA (12CA5)
from mouse $IgG_{2b}κ$

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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
Cat. No. 11 666 606 001 5 mg
1 ml

Store the product at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	Anti-HA (12CA5)	White lyophilizate	11 583 816 001	1 vial, 200 µg
		▪ Frozen liquid ▪ 5 mg/ml	11 666 606 001	1 vial, 1 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Anti-HA (12CA5) lyophilizate	Store at −15 to −25°C.
	Anti-HA (12CA5) solution	Store in aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

For reconstitution and dilution of Anti-HA

- PBS*
- Tween 20*

For western blotting

ⓘ *See section,* **Working Solution** *for additional information on preparing solutions.*
- Western blotting apparatus
- Western Transfer Buffer: 10% methanol, 24 mM Tris-base*, 194 mM glycine
- PVDF Membranes*
- PBS*
- Tween 20*
- Western Blocking Reagent*
- Secondary, Peroxidase-conjugated anti-mouse antibody
- Lumi-Light Western Blotting Substrate* or Lumi-Light^{PLUS} Western Blotting Substrate*
- Lumi-Film Chemiluminescent Detection Film*

1.4. Application

Anti-HA (12CA5) allows 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
- Immunocytochemistry

Use Anti-HA High Affinity* rat monoclonal antibody for higher sensitivity detection in western blotting.
ⓘ *10-fold lower concentration is needed.*

2. How to Use this Product

2.1. Before you Begin

General Considerations

Epitope tagging

Before using Anti-HA to analyze the product of your target gene, incorporate the 27-base DNA sequence that encodes the HA epitope into the target gene sequence by one of the following methods:
- Clone the 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.
- Insert the HA-peptide coding sequence into the target gene by oligonucleotide-mediated site-directed mutagenesis.

Working Solution

Solution	Composition/Preparation	Storage and Stability	For use in...
Anti-HA (12CA5) stock solution	Add 500 µl PBS* to the lyophilizate to a final concentration of 0.4 mg/ml.	Store in aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***	Anti-HA (12CA5) working solution.
Anti-HA (12CA5) working solution	Dilute the Anti-HA (12CA5) stock solution with Blocking solution B to a final concentration of 0.1 to 1.0 µg/ml.	–	Detection
Anti-POD-conjugated antibody	Dilute the secondary, POD-conjugated antibody with Blocking solution B to a final concentration of 1:10,000.	–	Detection
Washing buffer (PBST)	Dilute Tween 20* to 0.05 to 2% (v/v) final concentration in PBS, pH 7.5.	–	Washing
Blocking solution A	1x PBST, containing 10% (w/v) Western Blocking Reagent*.	–	Blocking
Blocking solution B	1x PBST, containing 5% (w/v) Western Blocking Reagent*.	–	Anti-HA (12CA5) working solution and Anti-POD-conjugated antibody.

2.2. Protocols

Western blotting

The following procedure describes the detection of a HA-tagged protein by enzyme-mediated chemiluminescence for a 10 × 10 cm membrane. 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*.

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2. Block the membrane with 10 ml Blocking solution A for 1 hour at +15 to +25°C or overnight at +2 to +8°C.
   – Place the container on a rotating platform.
   ⚠ ***Make sure the reagent completely and constantly covers the membrane during all incubation steps.***

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3. Drain the Blocking solution from the container.
   – Wash the membrane once with Washing buffer.

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4. Incubate the membrane with Anti-HA (12CA5) working solution for 1 hour at +15 to +25°C under gentle rotation.

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5. Drain the antibody solution.
   – Rinse the membrane with 20 ml Washing buffer.
   – Wash 4 × 10 minutes with 20 ml Washing buffer.

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6. Incubate the membrane with Anti-POD-conjugated antibody for 1 hour at +15 to +25°C under gentle rotation.

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7. Wash 4 × 10 minutes with Washing buffer.
   – Prepare Lumi-Light Western Blotting Substrate* according to the Instructions for Use.

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8. Drain the antibody solution.
   – Rinse the membrane with 20 ml Washing buffer.
   – Wash 4 × 10 minutes with 20 ml Washing buffer.

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9. Add the Lumi-Light reagent to the membrane.

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10. Expose the membrane to X-ray film or Lumi-Film*.
    ⓘ *For a 1 minute substrate development, perform a 1 to 5 minute exposure initially. The conditions for development and exposure may vary.*

Immunoprecipitation and immunoaffinity purification

Anti-HA antibody is commonly used to isolate HA-tagged proteins and associated protein:protein binding partners by various immunoprecipitation or immunoaffinity purification methods.

- For best results, the conditions for each application may vary and are to be determined individually.
- Use Anti-HA Affinity Matrix* (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 both 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. Optimal conditions for staining and fluorescent microscopy are to be determined for each particular application and method. The recommended starting concentration is 1 to 10 µg/ml.

ⓘ *For direct immunofluorescent detection, use anti-HA-Fluorescein, High Affinity (3F10)*.*

2.3. Parameters

Affinity/Binding Capacity

$K_a = 1 \times 10^8$/M

Isoelectric Point

6.4

Purity

≥90%
The anti-HA monoclonal antibody is ≥90% pure as determined by SDS-PAGE and HPLC.
The antibody preparation does not contain any preservatives or stabilizers.

Specificity

Anti-HA recognizes the HA nonapeptide sequence YPYDVPDYA derived from the human influenza virus hemagglutinin protein (amino acids 98 to 106). It recognizes its antigenic determinant even when the HA peptide epitope is introduced into unrelated recombinant proteins using a technique known as epitope tagging.

3. Troubleshooting

Observation	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.
		Eliminate all 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.	Prepare 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, or 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.	Prolong wash time.
	Incubation of membrane with substrate too long.	Leave blot membrane in substrate for a shorter time.
	Wrong membrane type.	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. ⚠ ***Do not touch membranes with bare hands; always use gloves and forceps.***
	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

4.1. Test Principle

Background information

The Anti-HA antibody (12CA5) 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.

Preparation

① Anti-HA 12CA5 is a subclone of H26DO8. The 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 (KLH).

② Spleen cells were isolated and fused with SP2/0 myeloma cells in polyethylene glycol to create the H26DO8 hybridoma clone.

③ The 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).

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	
ℹ *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

Editorial changes.
Layout changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Anti-HA-Fluorescein, High Affinity	25 µg	11 988 506 001
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 cm²	11 921 673 001
	6 x 100 ml, 60 blots, 100 cm²	11 921 681 001
Tris base	1 kg, *Not available in US*	10 708 976 001
	1 kg	03 118 142 001
	5 kg	11 814 273 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Lumi-Film Chemiluminescent Detection Film	100 films, 7.1 x 9.4 inches, 18 x 24 cm, *Not available in US*	11 666 916 001
	100 films, 8 x 10 inches, 20.3 x 25.4 cm	11 666 657 001
Lumi-Light Western Blotting Substrate	1 kit, 4,000 cm² membrane, 400 blots with 10 x 10 cm	12 015 200 001
Lumi-Light^{PLUS} Western Blotting Substrate	1 kit, 1,000 cm² membrane, 100 blots with 10 x 10 cm	12 015 196 001
Anti-HA Affinity Matrix	1 ml, settled resin volume	11 815 016 001
Anti-HA High Affinity	50 µg	11 867 423 001
	500 µg	11 867 431 001

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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