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

Anti-HA-Peroxidase

from mouse $IgG_{2b}\kappa$ (clone 12CA5)

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Peroxidase-conjugated mouse monoclonal antibody (clone 12CA5) to a peptide epitope derived from the hemagglutinin protein of human influenza virus.

Cat. No. 11 667 475 001	50 µg 500 µl

Store the product at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Anti-HA-Peroxidase	▪ Mouse monoclonal antibody clone 12CA5. ▪ 0.1 mg/ml	1 vial, 500 µl

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-Peroxidase	Prepare aliquots and store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

For western blotting

- 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 to 2% Tween 20*, pH 7.5
- Blocking solution 1:10 dilution of Western Blocking Reagent* in 1x PBST
- Lumi-Light Western Blotting Substrate* or Lumi-Light^{PLUS} Western Blotting Substrate*
- Lumi-Film Chemiluminescent Detection Film*

1.4. Application

Anti-HA-Peroxidase is used for the immunochemical detection of native influenza hemagglutinin protein and recombinant epitope-tagged proteins that contain the HA epitope in western and dot blots. Use of Anti-HA-Peroxidase eliminates the need for a secondary detection step.

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

2. How to Use this Product

2.1. Before you Begin

General Considerations

Epitope tagging

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

Working Solution

The Anti-HA-Peroxidase preparation is ready to use as supplied. For western blotting analysis using a 10 × 10 cm membrane, dilute 10 µl of undiluted Anti-HA-Peroxidase in 10 ml of a 1:20 dilution of Western Blocking Reagent in PBST immediately prior to use.

⚠ ***Do not add sodium azide to any reagent used in the procedure. Azide inhibits the enzymatic activity of POD.***

2.2. Protocols

Western blot analysis

ℹ *See section,* **Additional Equipment and Reagent Required** *for information on preparing solutions.*

1. Perform electrophoresis according to standard protocols.
   – Pre-wet a PVDF membrane* (0.45 µm pore size) in 100% methanol, and subsequently equilibrate the PVDF membrane for at least 5 minutes in Western transfer buffer.
   – Perform western blot transfer to the PVDF membrane using Western transfer buffer.
   ⚠ ***The buffer must be at +2 to +8°C for electrophoretic transfer.***

2. 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.***
   ℹ *A disposable, square Petri dish (100 × 15 mm) makes a convenient container for a 10 × 10 cm blot.*

3. After transfer, block the membrane for 1 hour at +15 to +25°C with 10 ml[(1)] of Blocking solution.
   – Place container on a rotating platform and rotate gently for 1 hour at +15 to +25°C.
   ⚠ ***Make sure the reagent completely and constantly covers the membrane during this incubation and all other incubation steps.***
   ℹ *This incubation step may also be performed at +2 to +8°C overnight.*

4. Drain the Blocking solution from the container and wash the blocked membrane once with PBST.

5. Thaw the undiluted Anti-HA-Peroxidase and store on ice prior to use.
   – Dilute 10 µl of undiluted Anti-HA-Peroxidase in 10 ml of a 1:20 dilution of Western Blocking Reagent in PBST immediately prior to use.
   – Incubate blocked membrane with diluted Anti-HA-Peroxidase for 1 hour at +15 to +25°C with gentle rotation.

6 Drain the antibody solution from the container and rinse the membrane with approximately 20 ml[1] of Wash buffer.
– Wash the membrane four times, 10 minutes per wash, with 20 ml[1] PBST.

7 Prepare a Detection solution according to the protocol described in the Lumi-Light reagent Instructions for Use.
– Add the Lumi-Light reagent to the membrane.

8 Drain excess Detection solution from the membrane, and wrap the blot in plastic wrap.
– Expose the membrane to X-ray film, such as Lumi-Film*, in a film cassette. For a 1 minute substrate development, perform a 1 to 5 minutes exposure initially.
Conditions for substrate development and X-ray film exposure needed to achieve optimum signal may vary with each experiment.

[1] Volume required per 100 cm^2 (10 × 10 cm) membrane.

2.3. Parameters

Affinity/Binding Capacity

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

Isoelectric Point

6.4

Purity

≥90% as determined by HPLC.

The antibody preparation does not contain preservatives or stabilizers.

Specificity

Anti-HA-Peroxidase recognizes the HA nonapeptide sequence YPYDVPDYA derived from the human influenza virus hemagglutinin protein (amino acids 98 to 106). 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.

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-Peroxidase.
	Too little protein on the gel.	Add more protein to gel.
	Poor transfer of proteins from gel to membrane.	Verify efficiency of protein transfer from gel to membrane by silver staining the remaining gel.
		To improve transfer efficiency, 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-Peroxidase 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 blotting 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, ot 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-Peroxidase by half.
	Wash time too short.	Extend wash time.
	Incubation of membrane with substrate too long.	Leave blot membrane in substrate for a shorter time.
	Wrong type of membrane.	For minimum background, use PVDF membranes* for transfer.
	Blocking reagent too dilute.	Use nonfat dry milk (5% w/v) dissolved in PBST as Blocking solution and antibody diluent. ⚠ ***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.***
	Signal development time too long.	Reduce development time by half.
	Additional bands.	Cross-reacting bands have been reported in certain western blot experiments performed with Anti-HA. In order to determine the specificity of the anti-HA-Peroxidase, include a negative-control cell extract prepared from the host organism and lacking the HA-tagged protein being analyzed.

4. Additional Information on this Product

4.1. 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.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
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
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
Lumi-Light^{PLUS} Western Blotting Substrate	1 kit, 1,000 cm^2 membrane, 100 blots with 10 x 10 cm	12 015 196 001
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
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-Light Western Blotting Substrate	1 kit, 4,000 cm^2 membrane, 400 blots with 10 x 10 cm	12 015 200 001
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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Roche

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany