Anti-GAPDH

Store at –20°C.
Do not store in a frost-free freezer.

Catalog #:	AM4300
Amount:	100 µg
Product Description:	Mouse monoclonal antibody to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).
Suggested Working Concentrations:	For immunofluorescent stain: 5 µg/mL For Western analysis: 1 µg/mL
Specificity:	Reacts with fish, frog, chicken, rabbit, mouse, human, and rat GAPDH, does not react with yeast GAPDH.
Clone:	6C5
Ig Isotype:	IgG_1
MW of Antigen:	36 kDa
Host Animal:	Mouse. Hybridization of SP2/0 myeloma cells with spleen cells from Balb/c mice.
Immunogen:	Purified rabbit muscle GAPDH (whole molecule).
Purification:	Chromatography on protein A Sepharose. Purity is tested by SDS-PAGE.
Storage Conditions:	For short-term storage, store at 4°C. For long-term storage, store at –20°C. ***Avoid repeated freeze/thaw cycles. Do not store in a frost-free freezer.***
Storage Buffer:	PBS, pH 7.4, 0.1% sodium azide

USER INFORMATION

Warning: **This product contains sodium azide. When disposing of this reagent through lead or copper plumbing, flush with copious volumes of water to prevent azide build-up in drains.**

General Information: Ambion Anti-GAPDH, monoclonal antibody to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), can be used to analyze the GAPDH protein expression in experimental cell lines by immunofluorescence and Western blot. Applied Biosystems/Austin scientists have used the Anti-GAPDH antibody in combination with Ambion GAPDH siRNA control products to study the specificity and effectiveness of the RNA interference (RNAi) effect at the protein level. When analyzed using Western blot, the target protein runs between 37–40 kDa.

Applications: Common applications for Anti-GAPDH include detection of GAPDH protein by ELISA, Western blotting, and Immunofluorescence, and immunoaffinity purification of GAPDH protein.
The following protocols for immunofluorescence and Western blotting with Anti-GAPDH are based on studies with adherent cells grown in 24-well tissue culture plates.

Protocol for Immunofluorescence
Dilute Anti-GAPDH (primary antibody) in 1X PBS to a recommended final concentration of 5 µg/mL for use in Step 9.

1. Plate cells at 50–80% confluency into each well of a 24-well tissue culture plate containing a round 12 mm glass cover slip.
2. 24 hr after plating, aspirate the culture medium from the dish and wash the cells with 1 mL of 1X PBS (Cat #AM9624, AM9625).
3. Into each well, add 400 µL of fresh 4% paraformaldehyde/PBS. (Prepare by weighing out 0.4 g paraformaldehyde powder and adding 10 mL of 1X PBS and 25 µL of 5 M NaOH. Heat to 65°C until dissolved, approximately 10 min, and then cool to room temperature before use.) Incubate for 7 min at room temperature with gentle agitation (use gentle agitation for all the incubation steps throughout the procedure).
4. Remove the paraformaldehyde and wash the cells with 1 mL of 1X PBS.
5. Remove PBS and add 500 µL of freshly prepared 0.1% Triton X-100/PBS, and incubate for 7 min to permeabilize cell membranes.
6. Remove Triton X-100/PBS and wash the cells with 1 mL of 1X PBS.
7. Remove PBS and block cells by adding 500 µL of 3% BSA/PBS (blocking solution) and incubate for 1 hr at room temperature with gentle agitation.
8. Remove the blocking solution and wash cells by adding 1 mL of 1X PBS.
9. Remove PBS, and add 500 µL of primary antibody diluted in 1X PBS and incubate for 1 hr at room temperature with gentle agitation.
10. Remove primary antibody (save for reuse) and wash the cells with 1 mL of 1X PBS.

Protect cells from light for the remainder of the protocol.

11. Remove PBS, add an appropriate secondary antibody conjugated with your choice of fluorescent marker (e.g., donkey anti-mouse IgG labeled with fluorescein [FITC]), and incubate for 1 hr at room temperature with gentle agitation.
12. Remove secondary antibody and wash the cells with 1 mL of 1X PBS.
13. Remove PBS and quickly wash the cells with 300 µL of nuclease-free water (e.g. Cat #AM9930).
14. Remove water.
15. Optional: For staining nuclei, include DAPI in the mounting media or include DAPI in the final 1X PBS wash and add an additional 1X PBS wash step prior to the water wash step. If desired, include an anti-fade agent in the mounting medium.
16. Mount the round-glass cover slip on a slide suitable for fluorescence microscopy.
17. Visualize protein by fluorescence microscopy using the appropriate fluorescence filters.

Protocol for Western Blot
Dilute Anti-GAPDH (primary antibody) in fresh blocking reagent for use in Step 15. The recommended final concentration for Anti-GAPDH antibody is 1 µg/mL. Diluted GAPDH antibody can be reused up to 3 times.

Sample Preparation

1. Collect the cells from a 24-well tissue culture plate using Trypsin-EDTA.
2. Transfer the cells from the plate to a 1.5 mL microcentrifuge tube.
3. Centrifuge the cells at 600 x g for 5 min, and remove the supernatant.
4. Wash with 500 µL 1X PBS.
5. Centrifuge the cells at 600 x g for 5 min, and remove the PBS.
6. Add 100 µL of lysis buffer (50 mM HEPES pH 8.3, 420 mM KCl, 0.1% NP-40, 1 mM EDTA).
7. Incubate for 15 min on ice.
8. Pellet cellular debris by centrifugation at 13,000 rpm at 4°C for 10 min.
9. Transfer the supernatant to a fresh microcentrifuge tube and keep it on ice for immediate use; alternatively store at –20°C.
10. Determine the total protein concentration.

Gel Electrophoresis and Western Blotting

11. Separate the proteins by SDS PAGE. The percentage of acrylamide in the gel and the amount of total protein loaded will vary depending on the size and abundance of the protein you are trying to examine.
12. Transfer the protein to a support membrane.
13. Block nonspecific binding by immersing the membrane in blocking reagent (e.g. 1% dry milk, 1X PBS) for 1 hr at room temperature with rocking.
14. Wash the membrane with PBST (0.1% Tween 20, 1X PBS) three times for 5 min each.
15. Add the primary antibody diluted in fresh blocking reagent to the membrane.
16. Incubate the membrane with the diluted primary antibody for 1 hr at room temperature with rocking.
17. Wash the membrane with PBST three times for 5 min each.
18. Dilute an appropriate secondary antibody conjugated to the enzyme or ligand of choice in fresh blocking reagent.
19. Incubate the membrane with the diluted secondary antibody for 1 hr at room temperature with rocking.
20. Wash the membrane with PBST three times for 5 min each.
21. Detect the protein level using an appropriate detection method for the conjugated secondary antibody.

For details about Western blotting, including appropriate membranes and detection systems, please see *Current Protocols in Molecular Biology* (John Wiley & Sons, Inc.).

For additional information on the procedure and protocol, please contact Ambion's Technical Services Department (1-800-888-8804, option 2 or techserv@ambion.com).

QUALITY CONTROL

Anti-GAPDH is rigorously tested for contaminating protease activity. Functionality is determined by immunofluorescence staining and Western blotting.

OTHER INFORMATION

Material Safety Data Sheets:

Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds. Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

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