

# Certificate of Analysis

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## Anti-GAPDH

Product No.	<b>AM4300</b>
Lot No.	<b>00939504</b>
Date of Manufacture	<b>2020-07-29</b>
Antibody Lot No.	<b>00939504</b>
Amount	100 µg
Product Description	Mouse monoclonal antibody to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).
Concentration	<b>8.2 mg/ml</b>
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 <sub>1</sub>
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. <b>Avoid repeated freeze/thaw cycles. Do not store in a frost-free freezer.</b>
Storage Buffer	PBS, pH 7.4, 0.1% sodium azide
USER INFORMATION	
Warning	<b>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.</b>
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

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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).

TEST	SPECIFICATION	RESULT
<b>Protease Activity</b>		
	A sample is incubated with protease substrate and analyzed by fluorescence.	Pass
<b>Functional Testing</b>		
	Tested by immunofluorescence staining and Western blotting.	Pass
OTHER INFORMATION		
Safety Data Sheets	Safety Data Sheets (SDSs) are available from <a href="http://www.thermofisher.com/support">www.thermofisher.com/support</a>	
Limited Product Warranty	Thermo Fisher Scientific Inc. and/or its affiliate(s) warrant their products as set forth in the Life Technologies General Terms and Conditions of Sale found on Thermo Fisher Scientific website at <a href="http://www.thermofisher.com/termsandconditions">www.thermofisher.com/termsandconditions</a> .	

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