

Product Datasheet

Anti-GAPDH Antibody (orb389477)

Catalog Number	orb389477
Description	Anti-GAPDH Antibody
Species/Host	Rabbit
Reactivity	Gallus, Human, Monkey, Mouse, Rat, Zebrafish
Conjugation	Unconjugated
Tested Applications	FC, ICC, IF, IHC, WB
Immunogen	E.coli-derived human GAPDH recombinant protein (Position: N136-E335). Human GAPDH shares 95% and 94.5% amino acid (aa) sequence identity with mouse and rat GAPDH, respectively.
Form/Appearance	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Note	For research use only
Application notes	Western blot, 0.1-0.5µg/ml, Human, Monkey, Mouse, Rat, Chicken, Zebrafish Immunohistochemistry (Paraffin-embedded Section), 2-5µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry(Fixed), 1-3 µg/1x10 ⁶ cells, Human. Add 0.2ml of distilled water will yield a concentration of 500ug/ml
Isotype	Rabbit IgG
Clonality	Polyclonal
Antibody Type	Primary Antibody

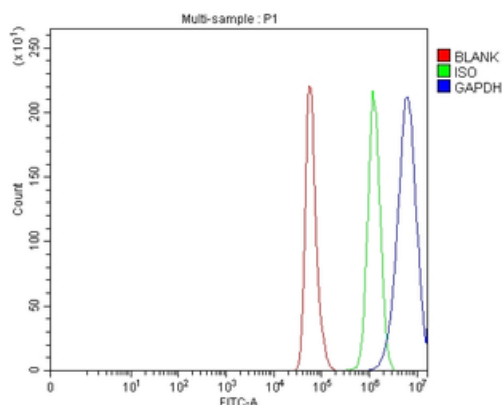
Biorbyt Ltd.

7 Signet Court, Swann's Road,
Cambridge, CB5 8LA, United Kingdom
Email: info@biorbyt.com, support@biorbyt.com
Phone: [+44 \(0\) 1223 859-353](tel:+44(0)1223859353) | Fax: [+1 \(415\) 651-8558](tel:+1(415)6518558)

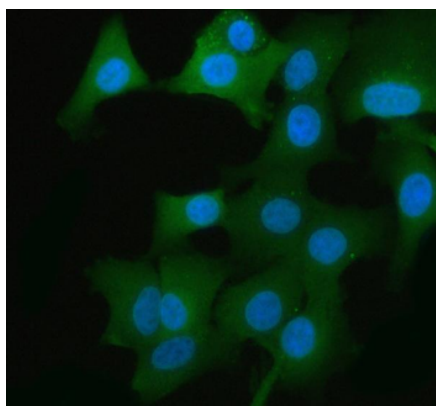
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Durham, NC, 27713, United States
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MW	36 kDa
Uniprot ID	P04406
Expiration Date	12 months from date of receipt.



Flow Cytometry analysis of Hela cells using anti-GAPDH antibody. Overlay histogram showing Hela cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-GAPDH Antibody (1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10⁶) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



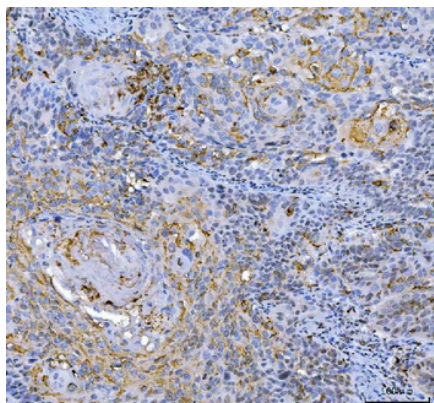
IF analysis of GAPDH using anti-GAPDH antibody. GAPDH was detected in an immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL rabbit anti-GAPDH Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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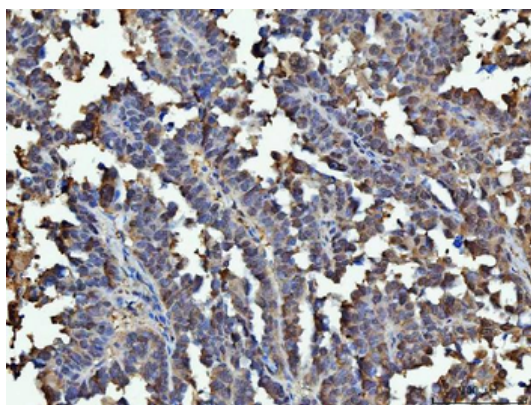
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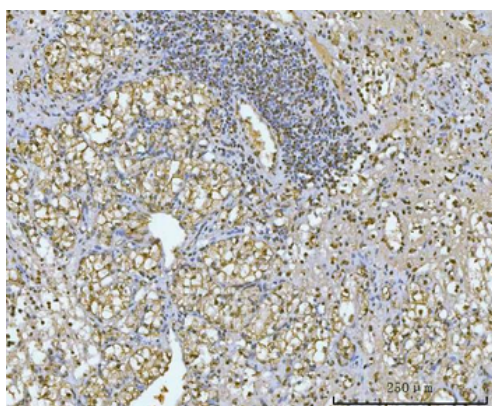
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IHC analysis of GAPDH using anti-GAPDH antibody. GAPDH was detected in a paraffin-embedded section of human laryngeal squamous cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-GAPDH Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



IHC analysis of GAPDH using anti-GAPDH antibody. GAPDH was detected in a paraffin-embedded section of human ovarian serous cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-GAPDH Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



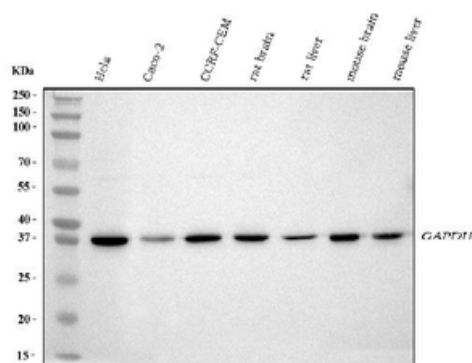
IHC analysis of GAPDH using anti-GAPDH antibody. GAPDH was detected in a paraffin-embedded section of human renal clear cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-GAPDH Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.

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Western blot analysis of GAPDH using anti-GAPDH antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human Hela whole cell lysates, Lane 2: human CACO-2 whole cell lysates, Lane 3: human CCRF-CEM whole cell lysates, Lane 4: rat brain tissue lysates, Lane 5: rat liver tissue lysates, Lane 6: mouse brain tissue lysates, Lane 7: mouse liver tissue lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-GAPDH antigen affinity purified polyclonal antibody at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1% Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with Tanon 5200 system. A specific band was detected for GAPDH at approximately 36 kDa. The expected band size for GAPDH is at 36 kDa.

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