

## Product Datasheet

### HADH Rabbit Polyclonal Antibody (orb1940013)

<b>Catalog Number</b>	orb1940013
<b>Category</b>	Antibodies
<b>Description</b>	Anti-HADH Antibody. Tested in ELISA, IF, IHC, ICC, WB, Flow Cytometry applications. This antibody reacts with Human, Mouse, Rat.
<b>Target</b>	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial
<b>Clonality</b>	Polyclonal
<b>Species/Host</b>	Rabbit
<b>Isotype</b>	IgG
<b>Conjugation</b>	Unconjugated
<b>Reactivity</b>	Human, Mouse, Rat
<b>Form/Appearance</b>	Lyophilized
<b>Concentration</b>	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
<b>Buffer/Preservatives</b>	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na <sub>2</sub> HPO <sub>4</sub> .
<b>Reconstitution</b>	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
<b>Purification</b>	Immunogen affinity purified.
<b>Immunogen</b>	E.coli-derived human HADH recombinant protein (Position: A43-K301). Human HADH shares 90.3% and 90.7% amino acid (aa) sequence identity with mouse and rat HADH, respectively.
<b>UniProt ID</b>	<b>Q16836</b>

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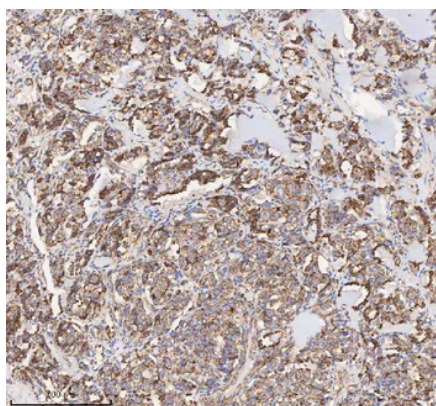
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<b>MW</b>	34 kDa
<b>Tested applications</b>	ELISA, FC, ICC, IF, IHC, WB
<b>Dilution range</b>	Western blot, 0.1-0.25 µg/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5 µg/ml, Human, Rat Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Immunofluorescence, 5 µg/ml, Human Flow Cytometry (Fixed), 1-3 µg/1x10 <sup>6</sup> cells, Human ELISA, 0.1-0.5 µg/ml
<b>Cross Reactivity</b>	No cross reactivity with other proteins.
<b>Antibody Type</b>	Primary Antibody
<b>Storage</b>	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.
<b>Note</b>	For research use only
<b>Expiration Date</b>	12 months from date of receipt.



IHC analysis of HADH using anti-HADH antibody. HADH was detected in a paraffin-embedded section of human lung adenocarcinoma 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-HADH 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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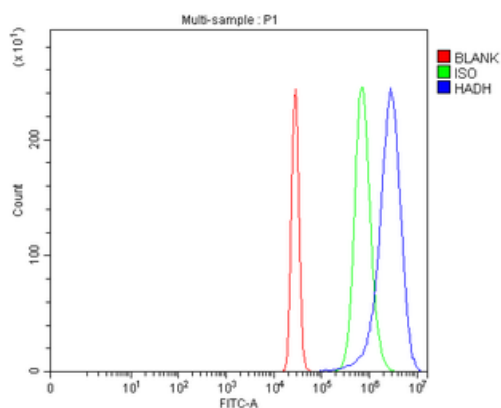
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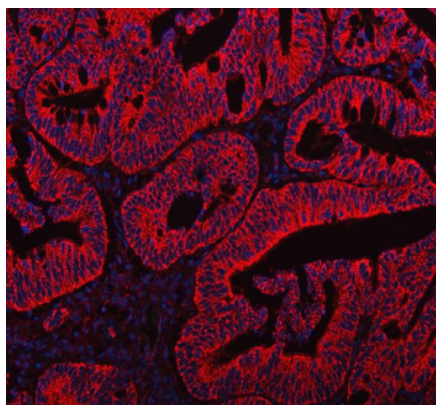
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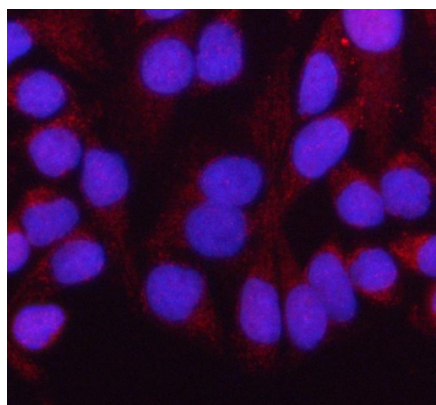
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Flow Cytometry analysis of HepG2 cells using anti-HADH antibody. Overlay histogram showing HepG2 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-HADH Antibody (1  $\mu\text{g}/1 \times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10  $\mu\text{g}/1 \times 10^6$  cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1  $\mu\text{g}/1 \times 10^6$ ) used under the same conditions. Unlabelled sample (Red line) was also used as a control.



IF analysis of HADH using antiHADH antibody. HADH was detected in a paraffin-embedded section of human intestinal 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 5  $\mu\text{g}/\text{mL}$  rabbit anti-HADH Antibody overnight at 4°C. Biotin conjugated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using DyLight®550 Conjugated Avidin. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IF analysis of HADH using anti-HADH antibody. HADH was detected in an immunocytochemical section of HELA 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  $\mu\text{g}/\text{mL}$  rabbit anti-HADH Antibody overnight at 4°C. Cy3 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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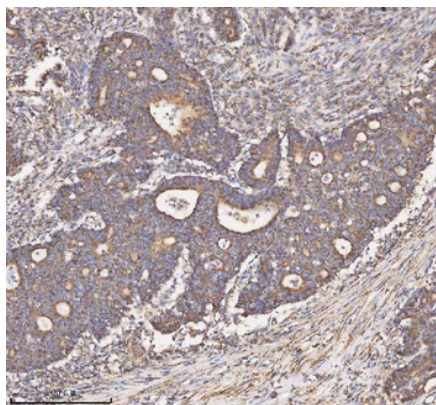
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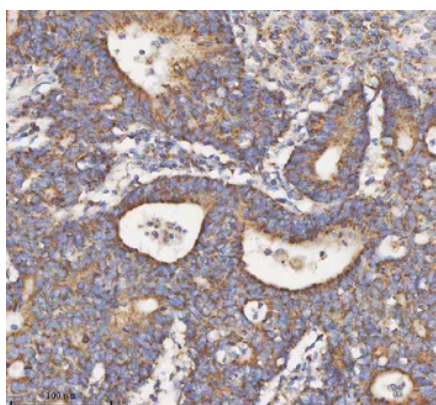
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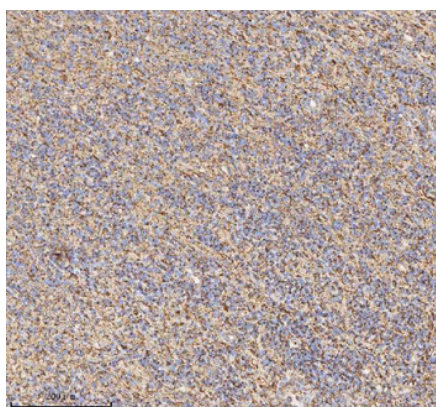
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IHC analysis of HADH using anti-HADH antibody. HADH was detected in a paraffin-embedded section of human colon adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu$ g/ml rabbit anti-HADH 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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IHC analysis of HADH using anti-HADH antibody. HADH was detected in a paraffin-embedded section of human glioblastoma 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  $\mu$ g/ml rabbit anti-HADH 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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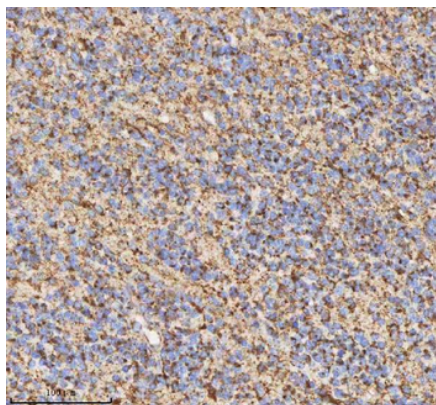
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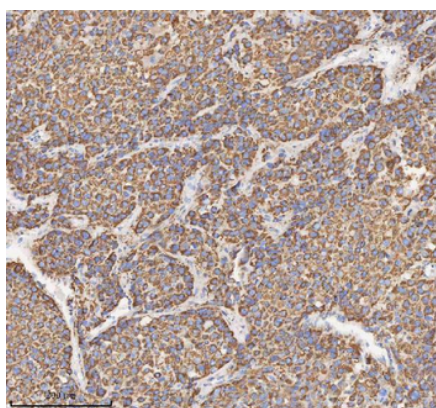
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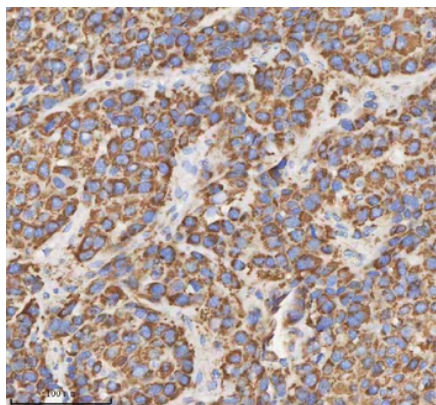
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IHC analysis of HADH using anti-HADH antibody. HADH was detected in a paraffin-embedded section of human glioblastoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-HADH 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 HADH using anti-HADH antibody. HADH was detected in a paraffin-embedded section of human liver 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-HADH 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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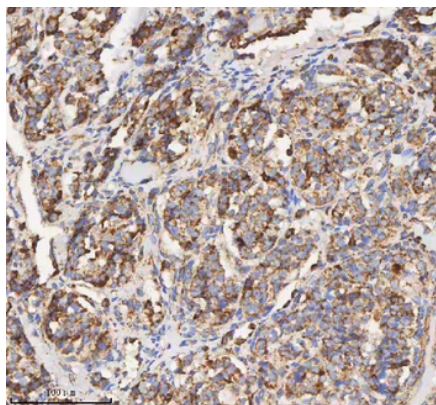
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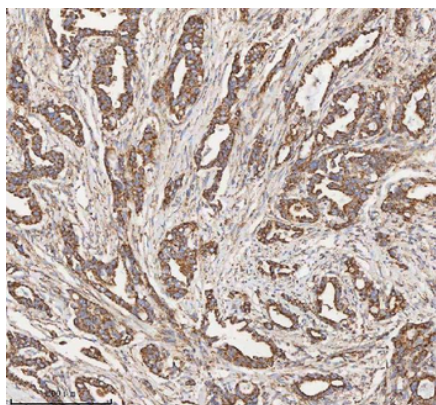
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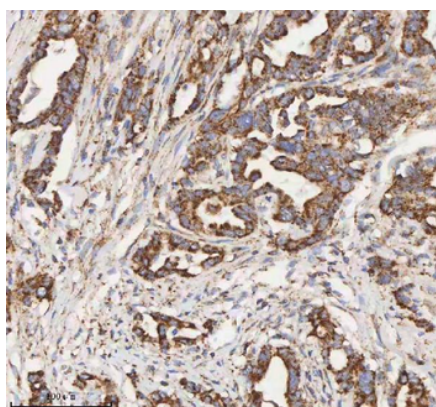
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IHC analysis of HADH using anti-HADH antibody. HADH was detected in a paraffin-embedded section of human pancreas ductal adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μg/ml rabbit anti-HADH 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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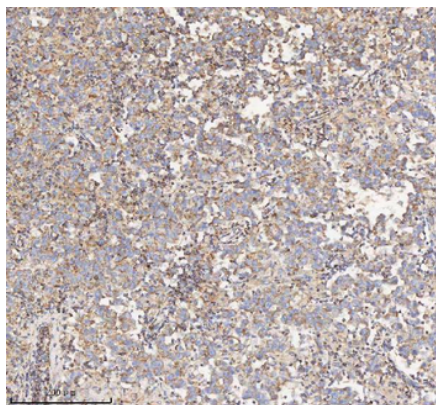
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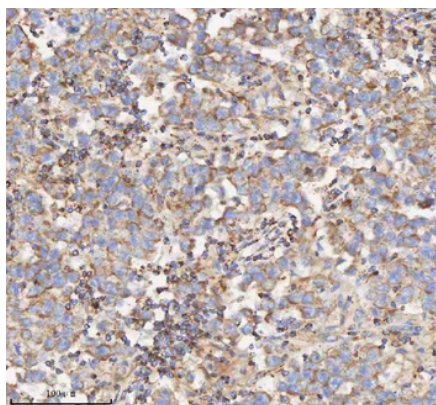
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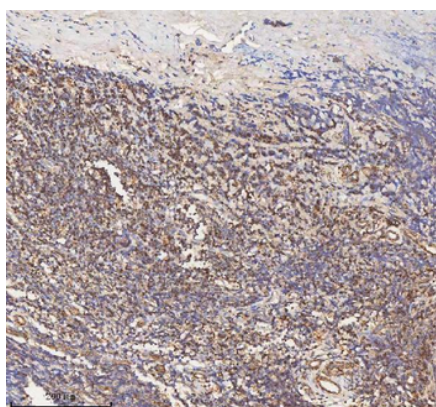
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IHC analysis of HADH using anti-HADH antibody. HADH was detected in a paraffin-embedded section of human testicular seminoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu$ g/ml rabbit anti-HADH 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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IHC analysis of HADH using anti-HADH antibody. HADH was detected in a paraffin-embedded section of human tonsil 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  $\mu$ g/ml rabbit anti-HADH 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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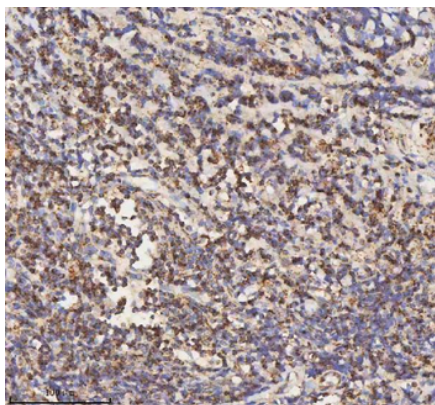
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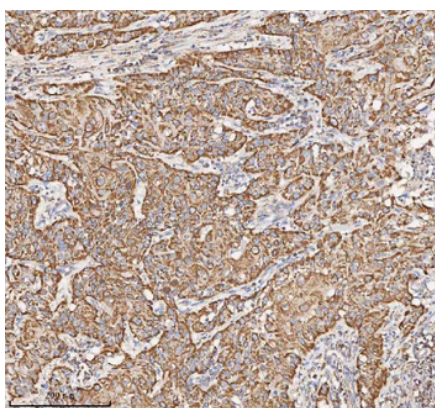
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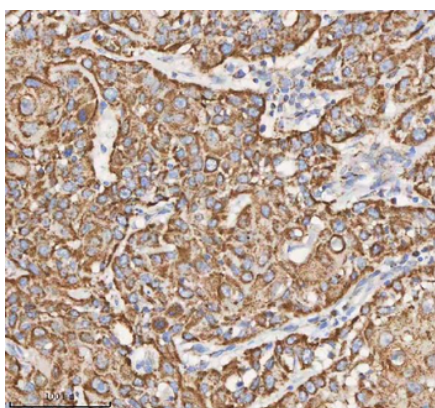
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IHC analysis of HADH using anti-HADH antibody. HADH was detected in a paraffin-embedded section of human tonsil tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-HADH 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 HADH using anti-HADH antibody. HADH was detected in a paraffin-embedded section of human urothelial 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-HADH 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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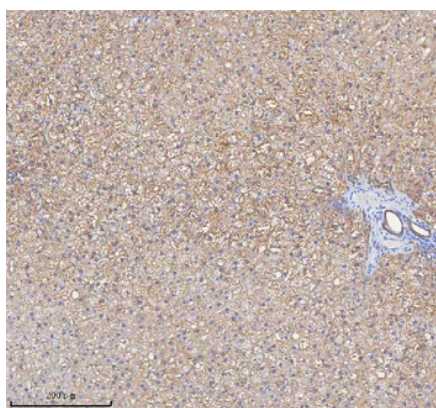
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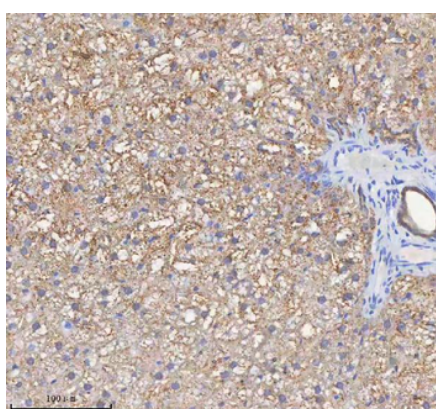
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IHC analysis of HADH using anti-HADH antibody. HADH was detected in a paraffin-embedded section of rat alcohol liver tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu\text{g/ml}$  rabbit anti-HADH 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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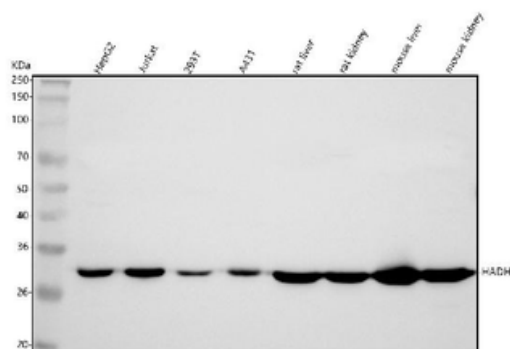
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Western blot analysis of HADH using anti-HADH antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human HepG2 whole cell lysates, Lane 2: human Jurkat whole cell lysates, Lane 3: human 293T whole cell lysates, Lane 4: human A431 whole cell lysates, Lane 5: rat liver tissue lysates, Lane 6: rat kidney tissue lysates, Lane 7: mouse liver tissue lysates, Lane 8: mouse kidney 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-HADH antigen affinity purified polyclonal antibody at 0.25  $\mu\text{g}/\text{mL}$  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 HADH at approximately 34 kDa. The expected band size for HADH is at 34 kDa.

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