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Anti-AKR1D1 Antibody Picoband™ (monoclonal, 6I4)

Catalog # ABO14952

Specification

Anti-AKR1D1 Antibody Picoband™ (monoclonal, 6I4) - Product Information

Application	**WB, IHC, FC**
Primary Accession	P51857
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-AKR1D1 Antibody Picoband™ (monoclonal, 6I4) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.

Reconstitution
Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Anti-AKR1D1 Antibody Picoband™ (monoclonal, 6I4) - Additional Information

Gene ID 6718

Other Names
Aldo-keto reductase family 1 member D1, 1.3.1.3, 3-oxo-5-beta-steroid 4-dehydrogenase, Delta(4)-3-ketosteroid 5-beta-reductase, Delta(4)-3-oxosteroid 5-beta-reductase, AKR1D1, SRD5B1

Calculated MW
37 kDa KDa

Application Details
Western blot, 0.1-0.5 µg/ml, Human, Mouse, Rat
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Subcellular Localization
Cytoplasm.

Tissue Specificity
Highly expressed in liver. Expressed in testis and weakly in colon.

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na₂HPO₄, 0.05mg NaN₃.

Immunogen
A synthetic peptide corresponding to a sequence at the C-terminus of human AKR1D1, which shares 90.9% and 93.9% amino acid (aa) sequence identity with mouse and rat AKR1D1, respectively.

Purification
Immunogen affinity purified.

Cross Reactivity
No cross-reactivity with other proteins.

Storage	**Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.**

Anti-AKR1D1 Antibody Picoband™ (monoclonal, 6I4) - Protein Information

Name AKR1D1

Synonyms SRD5B1

Function
Catalyzes the stereospecific NADPH-dependent reduction of the C4-C5 double bond of bile acid intermediates and steroid hormones carrying a delta(4)-3-one structure to yield an A/B cis-ring junction. This cis-configuration is crucial for bile acid biosynthesis and plays important roles in steroid metabolism. Capable of reducing a broad range of delta-(4)-3-ketosteroids from C18 (such as, 17beta- hydroxyestr-4-en-3-one) to C27 (such as, 7alpha-hydroxycholest-4-en-3- one).

Cellular Location
Cytoplasm.

Tissue Location
Highly expressed in liver. Expressed in testis and weakly in colon.

Anti-AKR1D1 Antibody Picoband™ (monoclonal, 6I4) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-AKR1D1 Antibody Picoband™ (monoclonal, 6I4) - Images

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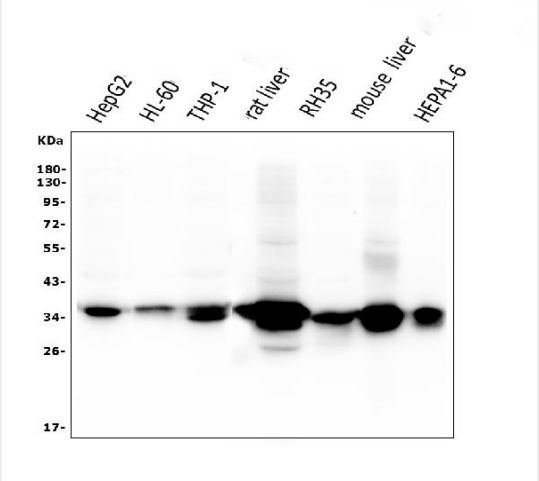

Figure 1. Western blot analysis of AKR1D1 using anti-AKR1D1 antibody (M05278).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human HepG2 whole cell lysates;
Lane 2: human HL-60 whole cell lysates;
Lane 3: human THP-1 whole cell lysates;
Lane 4: rat liver tissue lysates;
Lane 5: rat RH35 whole cell lysates;
Lane 6: mouse liver tissue lysates;
Lane 7: mouse HEPA1-6 whole cell lysates;
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-AKR1D1 antigen affinity purified monoclonal antibody (Catalog # M05278) 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-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for AKR1D1 at approximately 37KD. The expected band size for AKR1D1 is at 37KD.

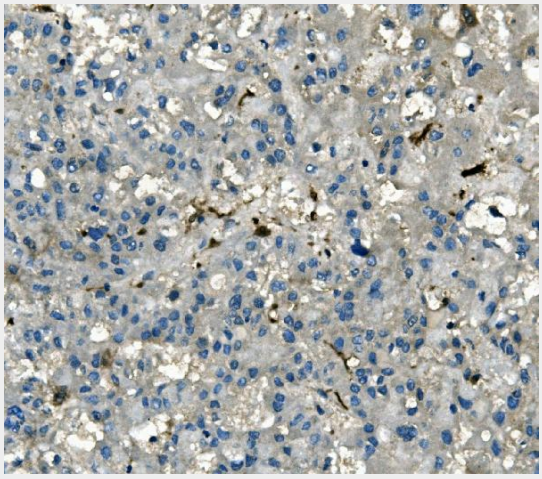

Figure 2. IHC analysis of AKR1D1 using anti-AKR1D1 antibody (M05278).
AKR1D1 was detected in paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-AKR1D1 Antibody (M05278) overnight at 4°C. Biotinylated goat anti-mouse IgG was

used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

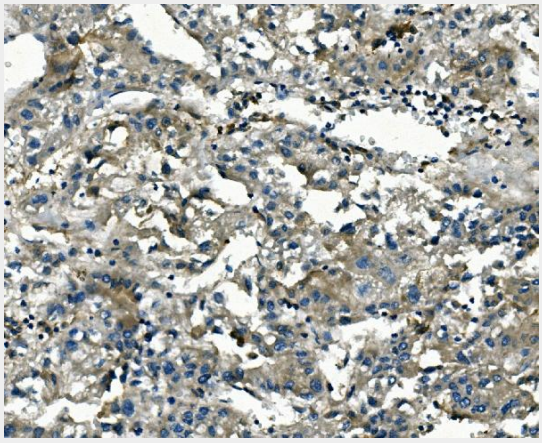

Figure 3. IHC analysis of AKR1D1 using anti-AKR1D1 antibody (M05278).
AKR1D1 was detected in paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-AKR1D1 Antibody (M05278) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

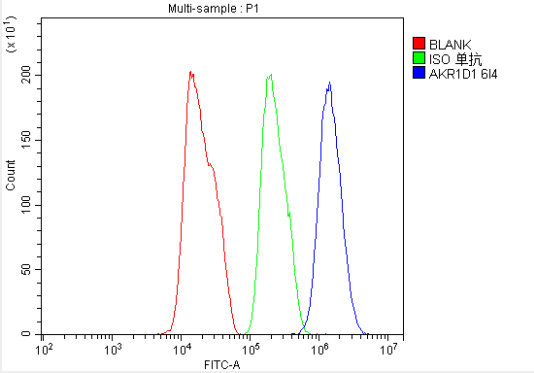

Figure 4. Flow Cytometry analysis of HepG2 cells using anti-AKR1D1 antibody (M05278).
Overlay histogram showing HepG2 cells stained with M04586-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-AKR1D1 Antibody (M05278,1 µg/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x106) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Anti-AKR1D1 Antibody Picoband™ (monoclonal, 6I4) - Background

Human delta (4)-3-oxosteroid 5-beta-reductase (steroid 5-beta-reductase) catalyzes 5-beta-reduction of bile acid intermediates and steroid hormones carrying a delta (4)-3-one structure. This gene is mapped to 7q33. The enzyme encoded by this gene is responsible for the catalysis of the 5-beta-reduction of bile acid intermediates and steroid hormones carrying a delta (4)-3-one structure. Deficiency of this enzyme may contribute to hepatic dysfunction. Three transcript variants encoding different isoforms have been found for this gene. Other variants may be present, but their full-length natures have not been determined yet.