

## Anti-ENO1 Antibody Picoband™

Catalog Number: A01250-1

### About ENO1

Enolase 1 (ENO1) is a glycolytic enzyme expressed in most tissues. It is mapped to 1p36.23. This gene encodes alpha-enolase, one of three enolase isoenzymes found in mammals. Each isoenzyme is a homodimer composed of 2 alpha, 2 gamma, or 2 beta subunits, and functions as a glycolytic enzyme. Alpha-enolase in addition, functions as a structural lens protein (tau-crystallin) in the monomeric form. Alternative splicing of this gene results in a shorter isoform that has been shown to bind to the c-myc promoter and function as a tumor suppressor. Several pseudogenes have been identified, including one on the long arm of chromosome 1. Alpha-enolase has also been identified as an autoantigen in Hashimoto encephalopathy.

### Overview

Product Name	Anti-ENO1 Antibody Picoband™
Reactive Species	Human, Monkey, Mouse, Rat
Description	Boster Bio Anti-ENO1 Antibody Picoband™ catalog # A01250-1. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na <sub>2</sub> HPO <sub>4</sub> , 0.05mg NaN <sub>3</sub> .
Storage Instructions	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.
Host	Rabbit
Uniprot ID	P06733

### Technical Details

Immunogen	A synthetic peptide corresponding to a sequence in the middle region of human ENO1, which shares 90.9% amino acid (aa) sequence identity with rat ENO1.
Predicted Reactive Species	Human
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti- Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.

Purification	Immunogen affinity purified.
Suggested Dilutions	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.1-0.25ug/ml, Human, Mouse, Monkey, Rat</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, Mouse, Rat</p> <p>Immunocytochemistry/Immunofluorescence, 2ug/ml, Human</p> <p>Flow Cytometry, 1-3ug/1x10<sup>6</sup> cells, Human</p>

## Anti-ENO1 Antibody Picoband™ (A01250-1) Images

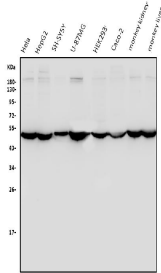


Figure 1. Western blot analysis of ENO1 using anti-ENO1 antibody (A01250-1).

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 Hela whole cell lysates,  
Lane 2: human HepG2 whole cell lysates,  
Lane 3: human SH-SY5Y whole cell lysates,  
Lane 4: human U-87MG whole cell lysates,  
Lane 5: human HEK293 whole cell lysates,  
Lane 6: human Caco-2 whole cell lysates,  
Lane 7: monkey kidney tissue lysates,  
Lane 8: monkey liver tissue 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 rabbit anti-ENO1 antigen affinity purified polyclonal antibody (Catalog # A01250-1) at 0.25 ug/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 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for ENO1 at approximately 47KD. The expected band size for ENO1 is at 47KD.

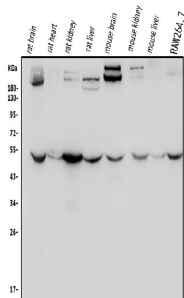


Figure 2. Western blot analysis of ENO1 using anti-ENO1 antibody (A01250-1).

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: rat brain tissue lysates,  
Lane 2: rat heart tissue lysates,  
Lane 3: rat kidney tissue lysates,  
Lane 4: rat liver tissue lysates,  
Lane 5: mouse brain tissue lysates,  
Lane 6: mouse kidney tissue lysates,  
Lane 7: mouse liver tissue lysates,  
Lane 8: mouse RAW264.7 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 rabbit anti-ENO1 antigen affinity purified polyclonal antibody (Catalog # A01250-1) at 0.25 ug/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 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for ENO1 at approximately 47KD.

The expected band size for ENO1 is at 47KD.

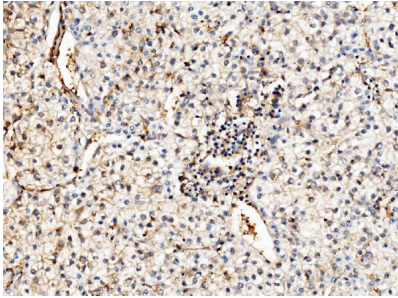


Figure 3. IHC analysis of ENO1 using anti-ENO1 antibody (A01250-1). ENO1 was detected in paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-ENO1 Antibody (A01250-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

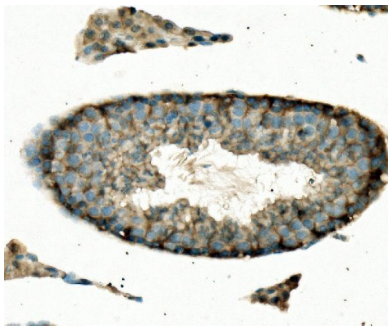


Figure 4. IHC analysis of ENO1 using anti-ENO1 antibody (A01250-1). ENO1 was detected in paraffin-embedded section of mouse testis tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-ENO1 Antibody (A01250-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

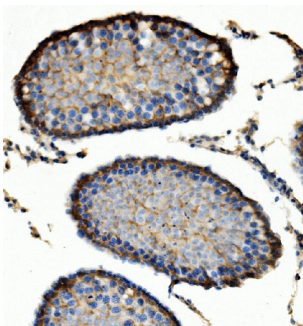


Figure 5. IHC analysis of ENO1 using anti-ENO1 antibody (A01250-1). ENO1 was detected in paraffin-embedded section of rat testis tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-ENO1 Antibody (A01250-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

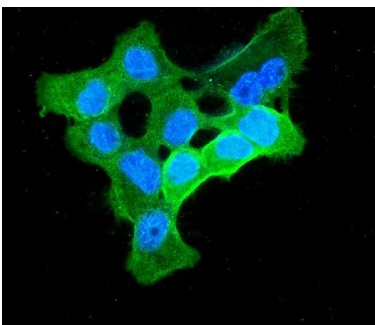


Figure 6. IF analysis of ENO1 using anti-ENO1 antibody (A01250-1). ENO1 was detected in immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL rabbit anti-ENO1 Antibody (A01250-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 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.

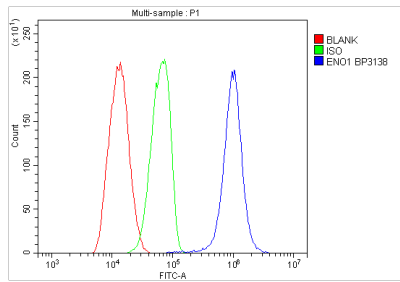


Figure 7. Flow Cytometry analysis of HL-60 cells using anti-ENO1 antibody (A01250-1). Overlay histogram showing HL-60 cells stained with A01250-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ENO1 Antibody (A01250-1, 1 $\mu$ g/1 $\times$ 10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 $\mu$ g/1 $\times$ 10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 $\mu$ g/1 $\times$ 10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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