Anti-Asparagine synthetase Antibody [SC67-07] ET1610-50

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Green monkey
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 64 kDa
Clone number:	SC67-07

Description: Asparagine synthetase (or aspartate-ammonia ligase) is a chiefly cytoplasmic enzyme that generates asparagine from aspartate. This amidation reaction is similar to that promoted by glutamine synthetase. The enzyme is ubiquitous in its distribution in mammalian organs, but basal expression is relatively low in tissues other than the exocrine pancreas. Cancerous cells exhibit rapid growth and cell division and subsequently have an increased nutritional need. The particularly low-level expression of asparagine synthetase in primary acute lymphoblastic leukemia (ALL) and numerous ALL cell lines, as compared to that of normal cells, makes asparagine depletion an effective method of treatment due to the cells' unusual dependency on circulating serum asparagine as a necessary nutrition for growth. A correlation between L-asparaginase efficacy and asparagine synthetase protein levels in a number of human ovarian cell lines has been observed. As mentioned above, this result confirmed similar observations in human leukemia cell lines. Hence asparagine synthetase might be used as a biomarker in ovarian cancer screening and potential treatment.

Immunogen: Synthetic peptide within Human Asparagine synthetase aa 502-538 / 561.

Positive control: K562 cell lysate, human skeletal muscle tissue lysate, human lung tissue lysates, K-562, mouse stomach tissue.

Subcellular location: Cytoplasm.

Database links: SwissProt: P08243 Human | Q61024 Mouse | P49088 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

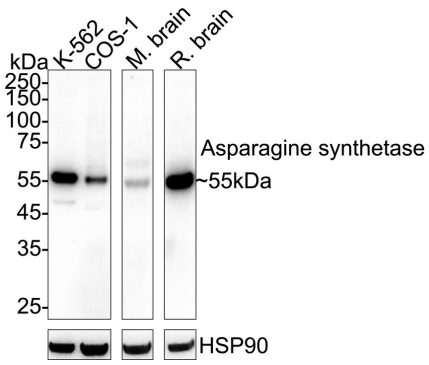

Fig1: Western blot analysis of Asparagine synthetase on different lysates with Rabbit anti-Asparagine synthetase antibody (ET1610-50) at 1/2,000 dilution.

Lane 1: K-562 cell lysate (20 µg/Lane)
Lane 2: COS-1 cell lysate (20 µg/Lane)
Lane 3: Mouse brain tissue lysate (40 µg/Lane)
Lane 4: Rat brain tissue lysate (40 µg/Lane)

Predicted band size: 64 kDa
Observed band size: 55 kDa

Exposure time: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1610-50) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

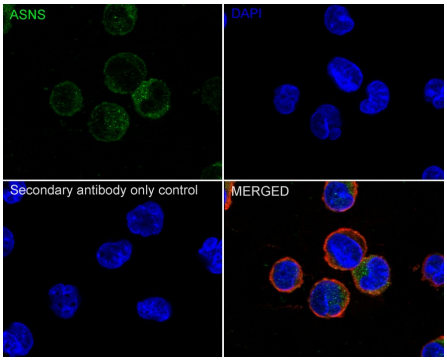

Fig2: Immunocytochemistry analysis of K-562 cells labeling Asparagine synthetase with Rabbit anti-Asparagine synthetase antibody (ET1610-50) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Asparagine synthetase antibody (ET1610-50) at 1/50 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

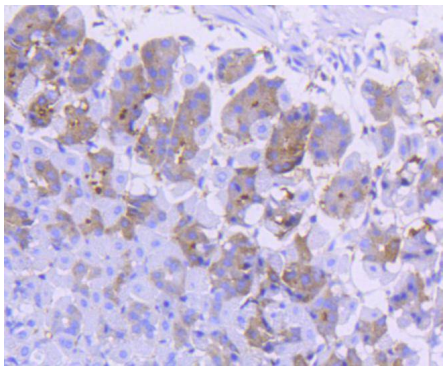

Fig3: Immunohistochemical analysis of paraffin-embedded mouse stomach tissue using anti-Asparagine synthetase antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-50, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Huang ML et al. Molecular and functional alterations in a mouse cardiac model of Friedreich ataxia: activation of the integrated stress response, eIF2a phosphorylation, and the induction of downstream targets. Am J Pathol 183:745-57 (2013).
2. Scotti C et al. Cell-cycle inhibition by Helicobacter pylori L-asparaginase. PLoS One 5:e13892 (2010).