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Anti-SHMT1 Antibody Picoband™ (monoclonal, 9C7)

Catalog # ABO15048

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P34896
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Rat, Human, Mouse, Monkey
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-SHMT1 Antibody Picoband™ (monoclonal, 9C7) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	6470
Other Names	Serine hydroxymethyltransferase, cytosolic, SHMT, 2.1.2.1, Glycine hydroxymethyltransferase, Serine methylase, SHMT1
Calculated MW	53083
Application Details	Western blot, 0.25-0.5 □g/ml, Mouse, Monkey, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5 □g/ml, Human Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 9C7
Immunogen	E.coli-derived human SHMT1 recombinant protein (Position: M1-S470).
Purification	Immunogen affinity purified.
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.

Protein Information

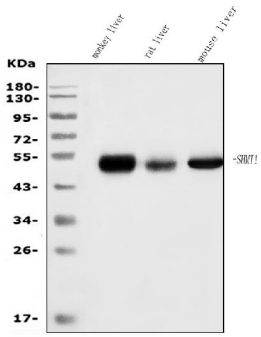
Name	SHMT1 (HGNC:10850)
Function	Pyridoxal phosphate (PLP)-dependent enzyme that catalyzes the reversible conversion of serine and tetrahydrofolate (THF) to glycine and 5,10-methylene THF, serving as a critical component of the folate cycle and facilitating one-carbon biosynthetic reactions essential for methionine, purine, and pyrimidine synthesis (PubMed:24698160, PubMed:30035852, PubMed:38996576). While its central activity involves serine cleavage, the detailed catalytic mechanisms remain under study, including both retro-aldol cleavage of the PLP-serine C(alpha)-C(beta) bond followed by formaldehyde condensation with THF, and alternative nucleophilic displacement mechanisms of the C(alpha) atom of PLP-serine aldimine involving THF's N5 atom (By similarity). Also catalyzes the cleavage of various 3-hydroxy amino acids, such as L-allo-threonine, L- threonine and 3-phenylserine, forming glycine and the corresponding aldehyde through a retro-aldol process; additionally, it catalyzes the formation of 5-formyltetrahydrofolate from 5,10-methenyltetrahydrofolate (PubMed:38615009). Also functions as a hydroxytrimethyllysine aldolase (HTMLA) catalyzing the second step of the carnitine biosynthesis pathway and exhibits substrate preference with the erythro (S,S) configuration, and more efficiency with L-allo- threonine (PubMed:38615009). In the nucleus, first functions as a lamin-binding scaffold protein that is essential for assembling the de novo thymidylate synthesis complex by co-localizing DHFR and TYMS with the nuclear lamina and anchoring the complex to DNA replication sites (PubMed:22235121). Subsequently, provides one-carbon substrates, specifically (6R)-5,10-methylene-5,6,7,8-tetrahydrofolate, in situ for de novo dTMP synthesis to sustain DNA replication and repair during cell proliferation (PubMed:30035852). Importantly, possesses RNA- binding capability, forming complexes that selectively regulate SHMT2 mRNA translation and dynamically modulate cytosolic and mitochondrial serine and glycine concentrations, thus influencing cellular metabolic status (PubMed:38996576).
Cellular Location	Cytoplasm. Nucleus. Note=Nuclear translocation is RAN- dependent and is independent from both the oligomerization state and the catalytic activity (PubMed:30035852). In the cytoplasm, SHMT1 degradation is facilitated by 'Lys-48'-linked ubiquitination, whereas in the nucleus, 'Lys-63'-linked ubiquitination prevents degradation (PubMed:22194612). Predominantly sumoylated and localized to the nucleus in S phase cells and remains in the nucleus during the G2/M phase of the cell cycle (PubMed:17446168). Sumoylation occurs at the nuclear pore and is linked to nuclear import (PubMed:17446168). As a component of the de novo thymidylate synthesis complex, localizes specifically to replication forks during DNA synthesis (PubMed:22235121).

Background

This gene encodes the cytosolic form of serine hydroxymethyltransferase, a pyridoxal phosphate-containing enzyme that catalyzes the reversible conversion of serine and tetrahydrofolate to glycine and 5,10-methylene tetrahydrofolate. This reaction provides one-carbon units for synthesis of methionine, thymidylate, and purines in the cytoplasm. This gene is located within the Smith-Magenis syndrome region on chromosome 17. A pseudogene of this gene is located on the short arm of chromosome 1. Alternative splicing results in multiple transcript variants.

Images

Figure 1. Western blot analysis of SHMT1 using anti-SHMT1 antibody (M02944).



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 30ug of sample under reducing conditions.
Lane 1: monkey liver tissue lysates,
Lane 2: rat liver tissue lysates,
Lane 3: mouse 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 mouse anti-SHMT1 antigen affinity purified monoclonal antibody (Catalog # M02944) 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 SHMT1 at approximately 53KD. The expected band size for SHMT1 is at 53KD.

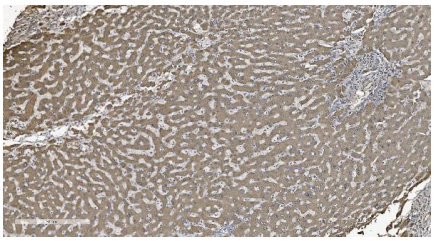


Figure 2. IHC analysis of SHMT1 using anti-SHMT1 antibody (M02944).
SHMT1 was detected in paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-SHMT1 Antibody (M02944) 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.

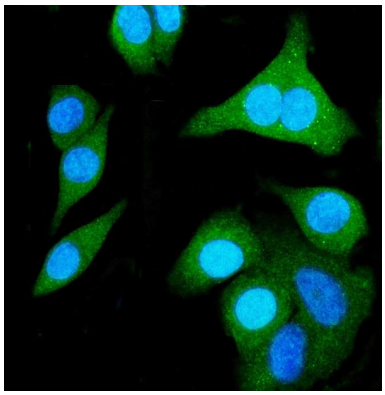


Figure 3. IF analysis of SHMT1 using anti-SHMT1 antibody (M02944).
SHMT1 was detected in immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-SHMT1 Antibody (M02944) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) 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.

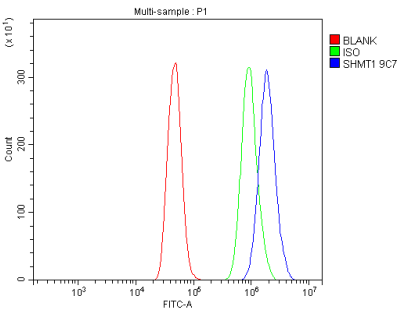


Figure 4. Flow Cytometry analysis of A431 cells using anti-SHMT1 antibody (M02944).
Overlay histogram showing A431 cells stained with M02944 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-SHMT1 Antibody (M02944, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody

(Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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