

Anti-ECSIT Antibody Picoband®

Catalog Number: A07966-3

About ECSIT

Evolutionarily conserved signaling intermediate in Toll pathway, mitochondrial (ECSIT), also known as SITPEC, is a protein that in humans is encoded by the ECSIT gene. Activation of NF- κ B as a result of Toll-like receptor (TLR) and IL-1 receptor signaling is a major component of innate immune responses. Signals from these receptors are relayed by a number of adapter molecules such as TRIF, TIRAP, and MyD88 to kinases such as IRAK and other intermediates such as TNF receptor associated factor (TRAF)-6. ECSIT (evolutionarily conserved signaling intermediate in Toll pathways) was initially identified as a cytoplasmic protein interacting specifically with TNF receptor associated factor (TRAF)-6 in the TLR pathway. Recently however, ECSIT has also been shown to be required for bone morphogenetic protein (Bmp) signaling and mesoderm formation during mouse embryogenesis, indicating the possibility of cross-talk between the TLR/IL-B and Bmp signaling pathways.

Overview

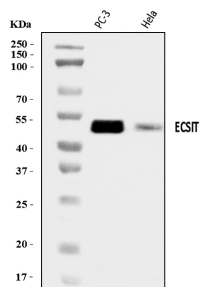
Product Name	Anti-ECSIT Antibody Picoband®
Reactive Species	Human
Description	Boster Bio Anti-ECSIT Antibody Picoband® catalog # A07966-3. Tested in ELISA, Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na ₂ HPO ₄ .
Storage Instructions	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 freezing and thawing.
Host	Rabbit
Uniprot ID	Q9BQ95

Technical Details

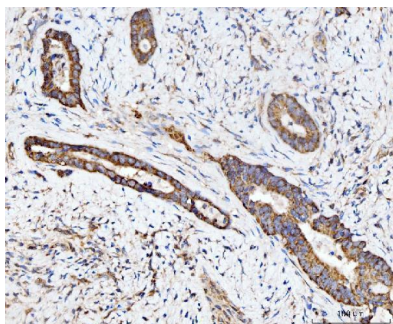
Immunogen	E.coli-derived human ECSIT recombinant protein (Position: E83-S339).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	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	Western blot, 0.1-0.25 ug/ml, Human Immunohistochemistry(Paraffin-embedded Section), 2-5 ug/ml, Human Immunocytochemistry/Immunofluorescence, 5 ug/ml, Human Flow Cytometry (Fixed), 1-3 ug/1x10 ⁶ cells, Human ELISA, 0.1-0.5 ug/ml, -

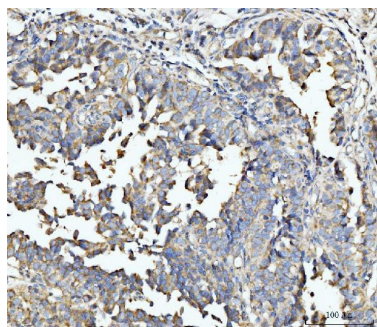
Anti-ECSIT Antibody Picoband® (A07966-3) Images



Western blot analysis of ECSIT using anti-ECSIT antibody (A07966-3). 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 PC-3 whole cell lysates, Lane 2: human HeLa whole cell 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-ECSIT antigen affinity purified polyclonal antibody (Catalog # A07966-3) 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 1:5000 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 ECSIT at approximately 49 kDa. The expected band size for ECSIT is at 49 kDa.

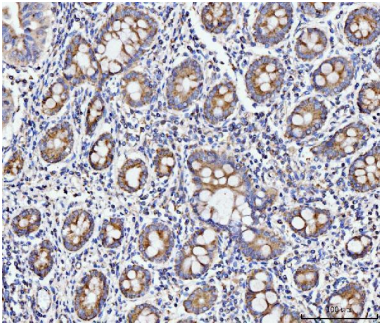


IHC analysis of ECSIT using anti-ECSIT antibody (A07966-3). ECSIT was detected in a paraffin-embedded section of human bladder adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-ECSIT Antibody (A07966-3) 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.

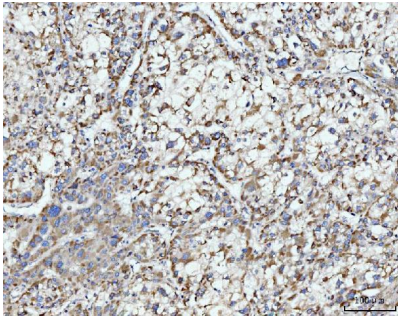


IHC analysis of ECSIT using anti-ECSIT antibody (A07966-3). ECSIT was detected in a paraffin-embedded section of human colonic adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-ECSIT Antibody (A07966-3) 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.

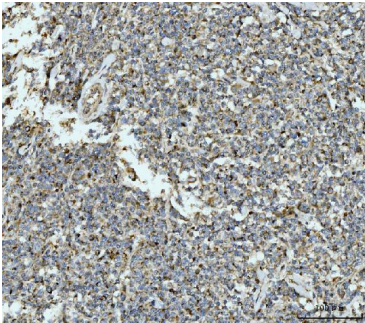
IHC analysis of ECSIT using anti-ECSIT antibody (A07966-3). ECSIT was detected in a paraffin-embedded section of human gastric adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated



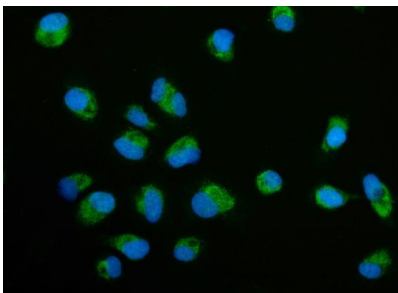
with 2 ug/ml rabbit anti-ECSIT Antibody (A07966-3) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



IHC analysis of ECSIT using anti-ECSIT antibody (A07966-3). ECSIT was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-ECSIT Antibody (A07966-3) 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.

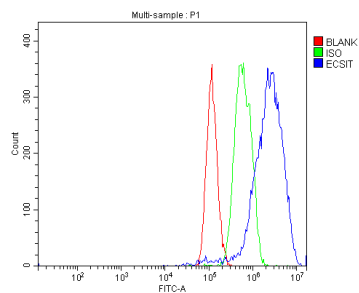


IHC analysis of ECSIT using anti-ECSIT antibody (A07966-3). ECSIT was detected in a paraffin-embedded section of human lymphadenoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-ECSIT Antibody (A07966-3) 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.



IF analysis of ECSIT using anti-ECSIT antibody (A07966-3). ECSIT was detected in an immunocytochemical section of PC-3 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 ug/mL rabbit anti-ECSIT Antibody (A07966-3) 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.

Flow Cytometry analysis of HepG2 cells using anti-ECSIT antibody (A07966-3). Overlay histogram showing HepG2 cells stained with A07966-3 (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-ECSIT Antibody



(A07966-3, 1 ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10⁶) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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Anti-ECSIT Antibody

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