

Anti-Caspase-9 Polyclonal Antibody

Product Details

Ig Type:	IgG
Reactivity:	Human,Mouse,Rat (predicted:Dog,Cow)
Molecular Weight:	Theoretical: 35/50 kDa. Actual: 52 kDa.
Purification:	Protein A purified

Applications

- Blank control: RSC96 (blue), the cells were fixed with 2% paraformaldehyde (10 min) and then permeabilized with ice-cold 90% methanol for 30 min on ice.
Isotype Control Antibody: Rabbit IgG (orange);
Secondary Antibody: Goat anti-rabbit IgG-Pe (white blue),
Dilution: 1:200 in 1 X PBS containing 0.5% BSA;
Primary Antibody Dilution: 1 µg in 100 µL 1X PBS containing 0.5% BSA (green).
 - Blank control: K562 (blue). Primary Antibody: Rabbit Anti-caspase-9 antibody (TMAB-00292, Green); Dilution: 1 µg in 100 µL 1X PBS containing 0.5% BSA; Isotype Control Antibody: Rabbit IgG (orange), used under the same conditions; Secondary Antibody: Goat anti-rabbit IgG-FITC (white blue), Dilution: 1:200 in 1 X PBS containing 0.5% BSA.
- Protocol
- The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.01M PBS-Tween for 20 min. Primary antibody (TMAB-00292, 1 µg/1x10⁶ cells) were incubated for 30 min at room temperature, followed by 1 X PBS containing 0.5% BSA + 10% goat serum (30 min) to block non-specific protein-protein interactions. Then the Goat Anti-rabbit IgG/FITC antibody was added into the blocking buffer mentioned above to react with the primary antibody at 1/200 dilution for 30 min at room temperature.
3. Paraformaldehyde-fixed, paraffin embedded (rat lung); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Insulin like growth factor 1) Polyclonal Antibody, Unconjugated at 1:400 overnight at 4°C, followed by a conjugated secondary antibody for 20 min and DAB staining.
4. Tissue/cell: Hela cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (Caspase-9) polyclonal Antibody, Unconjugated (TMAB-00292) 1:100, 90 minutes at 37°C; followed by a FITC conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nucleus.
5. Paraformaldehyde-fixed, paraffin embedded (rat pancreas); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Caspase-9) Polyclonal Antibody, Unconjugated (TMAB-00292) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
6. Tissue/cell: HepG2 cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (Caspase-9) polyclonal Antibody, Unconjugated (TMAB-00292) 1:100, 90 minutes at 37°C; followed by a FITC conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nucleus.

Verified Activity:

7. Tissue/cell: MCF-7 cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (Caspase-9) Polyclonal Antibody, Unconjugated (TMAB-00292) 1:50, 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nucleus.

8. HepG2 cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (Caspase-9) polyclonal Antibody, Unconjugated (TMAB-00292) 1:100, 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nucleus.

9. Sample:

293T-UV Cell (Human) Lysate at 30 µg

Primary: Anti-Caspase-9 at 1/300 dilution

Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution

Predicted band size: 35/50 kDa

Observed band size: 35/50 kDa

10. Sample:

Lane 1: Mouse Stomach tissue lysates

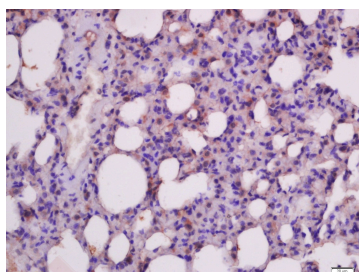
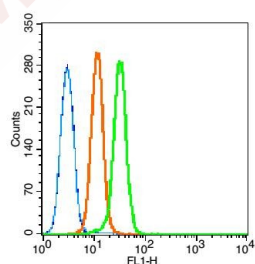
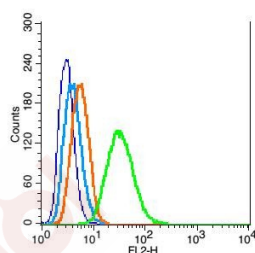
Lane 2: Mouse Spleen tissue lysates

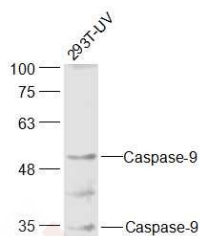
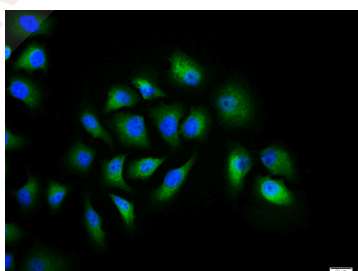
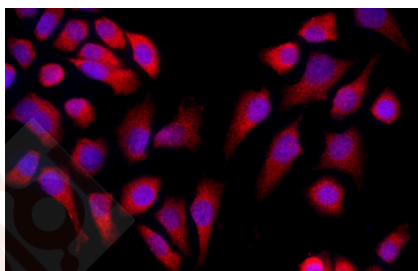
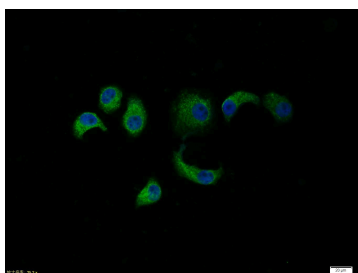
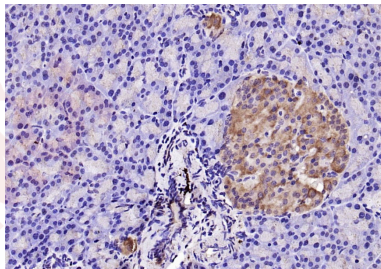
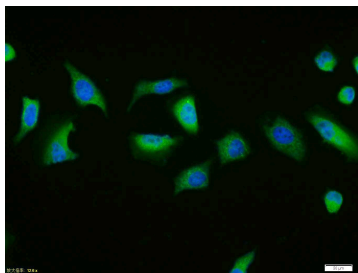
Primary: Anti-Caspase-9 (TMAB-00292) at 1/1000 dilution

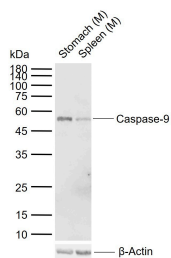
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution

Predicted band size: 35/50 kDa

Observed band size: 52 kDa







Application: FCM,ICC/IF,IF,IHC-Fr,IHC-P,WB

Recommended FCM=1 µg/Test; ICC/IF=1:100-500; IF=1:100-500; IHC-Fr=1:100-500; IHC-P=1:100-500; WB=1:500-2000

Properties

Stability & Storage: Store at -20°C or -80°C for 12 months. Avoid repeated freeze-thaw cycles.

Shipping: Shipping with blue ice.

Antigen Details

Immunogen: KLH conjugated synthetic peptide: human Caspase-9 subunit p35

Antigen Species: Human

Gene ID: 842

Uniprot ID: P55211

Synonyms: Caspase 9;CASP 9;Caspase 9 precursor;ICE like apoptotic protease 6;OTTHUMP00000044594;ICE LAP6;Apoptotic protease MCH6;RNCASP9;Apoptosis related cysteine peptidase;Caspase 9 apoptosis related cysteine protease;Caspase 9c;MCH6;Caspase-9 subunit p35;MCH 6;Caspase9;Apoptotic protease MCH 6;APAF 3;Apoptotic protease activating factor 3;APAF3;CASP9

Biology Area: Caspases,Cytochrome C,Metabolism,Caspases,Cytochrome C,Caspases,Apoptosis

Research Background

Caspase 9 (also known as ICE like apoptotic protease 6 (ICE LAP6), apoptotic protease Mch6, and apoptotic protease activating factor 3 (Apaf3)) is a member of the peptidase family C14 that contains a CARD domain. This caspase is active as a heterotetramer and has been reported to have two isoforms. ProCaspase 9 has been reported to be approximately 47 kD. This caspase is present in the cytosol and, upon activation, translocates to the mitochondria. Caspase 9 is involved in the caspase activation cascade responsible for apoptosis execution and cleaves/activates Caspase 3 and Caspase 6. Caspase 9 is inhibited by the dominant negative isoform, BclXL, cIAP1, cIAP2, XIAP, and Livin. This caspase becomes activated when recruited to Apaf1/cytochrome c complex, and following cleavage by Apaf1, granzyme B, Caspase 3, possibly Caspase 8 and Caspase 10 into large p37 and small p10 subunits. Caspase 9 interacts with BIRC7 and has been shown to cleave PARP and vimentin.

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Tel:781-999-4286 E_mail:info@targetmol.com Address:34 Washington Street,Wellesley Hills,MA 02481