

## Anti-Caspase-9 Polyclonal Antibody 3

## Product Details

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

## Applications

1. Paraformaldehyde-fixed, paraffin embedded (Rat heart); 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-00294) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
2. Paraformaldehyde-fixed, paraffin embedded (Rat urinary bladder); 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-00294) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
3. Sample:  
Spleen (Mouse) Lysate at 40 µg  
Primary: Anti-Caspase-9 (TMAB-00294) at 1/300 dilution  
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution  
Predicted band size: 35/50 kDa  
Observed band size: 35 kDa
4. Sample:  
Urinary bladder (Mouse) Lysate at 40 µg  
Jurkat (Human) Cell Lysate at 30 µg  
Primary: Anti-Caspase-9 (TMAB-00294) at 1/1000 dilution  
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution  
Predicted band size: 46-51/35/37 kDa  
Observed band size: 35 kDa
5. Paraformaldehyde-fixed, paraffin embedded (mouse 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-00294) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
6. 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-00294) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
7. HepG2 cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min;

Verified Activity:

Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (Caspase-9) polyclonal Antibody, Unconjugated (TMAB-00294) 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.

8. Sample: K562 Cell (Human) Lysate at 40 µg

Primary: Anti-Caspase-9 (TMAB-00294) at 1/300 dilution

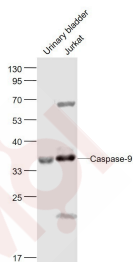
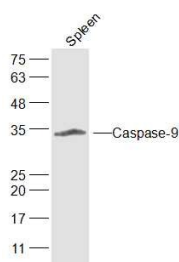
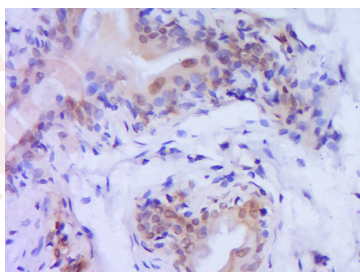
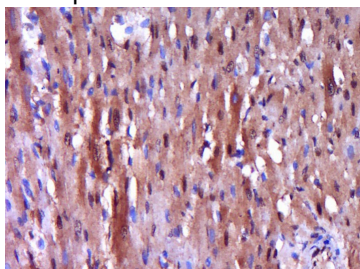
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution

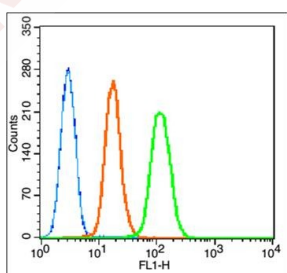
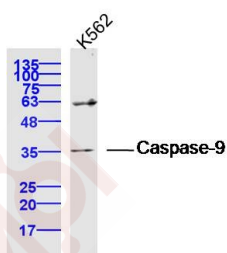
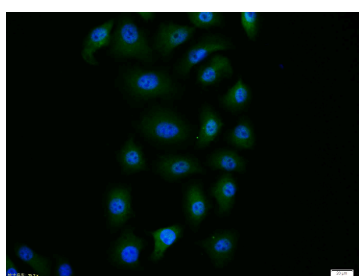
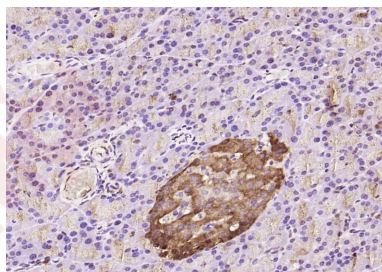
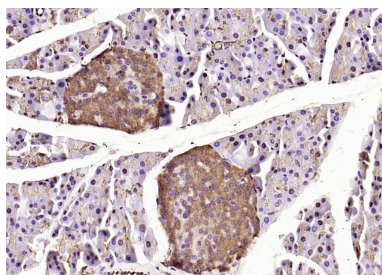
Predicted band size: 35/50 kDa

Observed band size: 35 kDa

9. Overlay histogram showing Hela cells (1 µg/1x10<sup>6</sup> cells) stained with TMAB-00294 (Green line).

The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.01M PBS-Tween for 20 min. The cells were then incubated in 1x PBS/10% normal goat serum to block non-specific protein-protein interactions followed by the antibody for 30 min at 22°C. The secondary antibody used was fluorescein isothiocyanate goat anti-rabbit IgG (H+L) at 1/200 dilution for 30 min at 22°C. Isotype control antibody was rabbit IgG (polyclonal, Orange line) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of 20,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.





Application: FCM, ICC/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
Antigen Species:	Human
Gene ID:	842
Uniprot ID:	P55211
Synonyms:	MCH6;MCH 6;Apoptotic protease activating factor 3;Caspase 9c;Apoptosis related cysteine peptidase;CASP 9;RNCASP9;Caspase 9 apoptosis related cysteine protease;Apoptotic protease MCH6;OTTHUMP0000044594;Caspase9;APAF 3;ICE like apoptotic protease 6;APAF3;CASP9; Apoptotic protease MCH 6;Caspase 9 precursor;ICE LAP6;Caspase-9 subunit p35;Caspase 9
Biology Area:	Caspases,Cytochrome C,Metabolism,Caspases,Cytochrome C,Caspases,Apoptosis

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### 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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