

## Product Datasheet

### Anti-Caspase-9/CASP9 Antibody (orb745902)

<b>Catalog Number</b>	orb745902
<b>Description</b>	Anti-Caspase-9/CASP9 Antibody. Tested in ELISA, Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
<b>Species/Host</b>	Rabbit
<b>Reactivity</b>	Human
<b>Conjugation</b>	Unconjugated
<b>Tested Applications</b>	ELISA, FC, ICC, IF, IHC, WB
<b>Immunogen</b>	E.coli-derived human Caspase-9/CASP9 recombinant protein (Position: E3-K410).
<b>Form/Appearance</b>	Lyophilized
<b>Concentration</b>	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
<b>Storage</b>	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
<b>Note</b>	For research use only
<b>Application notes</b>	Western blot, 0.25-0.5µg/ml, Human Immunohistochemistry (Paraffin-embedded Section), 2-5µg/ml, Human Immunocytochemistry/Immunofluorescence, 5µg/ml, Human Flow Cytometry (Fixed), 1-3µg/1x10 <sup>6</sup> cells, Human ELISA, 0.1-0.5µg/ml, -. Add 0.2ml of distilled water will yield a concentration of 500ug/ml
<b>Isotype</b>	Rabbit IgG
<b>Clonality</b>	Polyclonal
<b>Antibody Type</b>	Primary Antibody
<b>MW</b>	46 kDa

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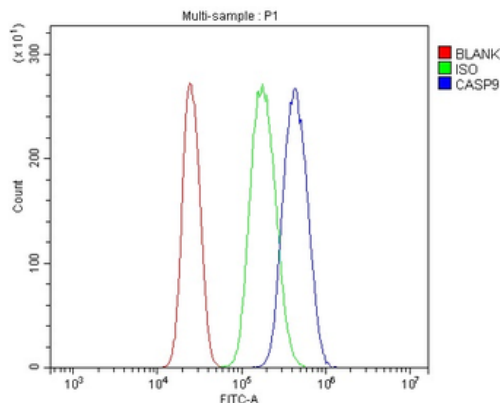
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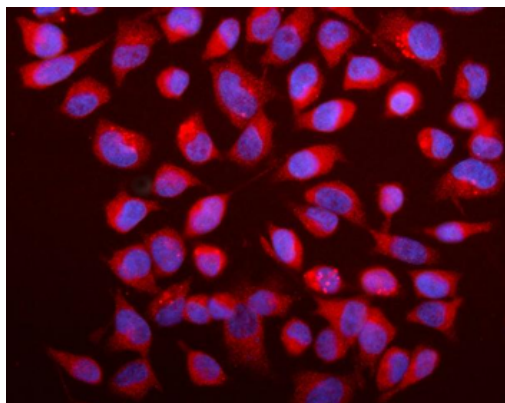
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**Uniprot ID****P55211****Expiration Date**

12 months from date of receipt.



Flow Cytometry analysis of U937 cells using anti-Caspase-9/CASP9 antibody. Overlay histogram showing U937 cells (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-Caspase-9/CASP9 Antibody (1  $\mu\text{g}/1 \times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10  $\mu\text{g}/1 \times 10^6$  cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1  $\mu\text{g}/1 \times 10^6$ ) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



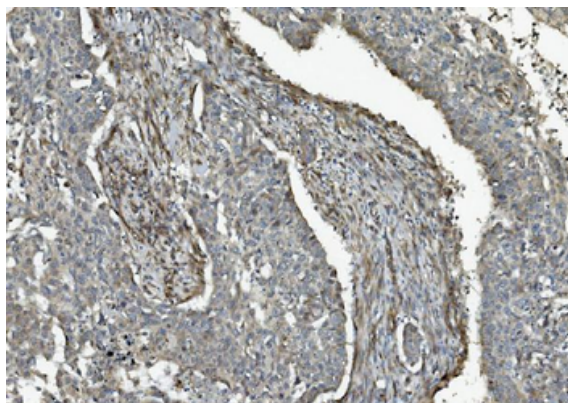
IF analysis of Caspase-9/CASP9 using anti-Caspase-9/CASP9 antibody. Caspase-9/CASP9 was detected in immunocytochemical section of HELA cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5  $\mu\text{g}/\text{mL}$  rabbit anti-Caspase-9/CASP9 Antibody overnight at 4°C. DyLight®594 Conjugated Goat Anti-Rabbit IgG 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.

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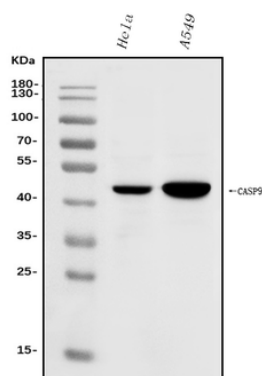
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IHC analysis of Caspase-9/CASP9 using anti-Caspase-9/CASP9 antibody. Caspase-9/CASP9 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Caspase-9/CASP9 Antibody 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) with DAB as the chromogen.



Western blot analysis of Caspase-9/CASP9 using anti-Caspase-9/CASP9 antibody. 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 µg of sample under reducing conditions. Lane 1: human HeLa whole cell lysates, Lane 2: human A549 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-Caspase-9/CASP9 antigen affinity purified polyclonal antibody 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-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 with Tanon 5200 system. A specific band was detected for Caspase-9/CASP9 at approximately 46 KD. The expected band size for Caspase-9/CASP9 is at 46 KD.

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