

Product Datasheet

Caspase-9 Antibody (LAP6 96-2-22) - BSA Free NB500-209

Unit Size: 0.2 mg

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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NB500-209

Caspase-9 Antibody (LAP6 96-2-22) - BSA Free

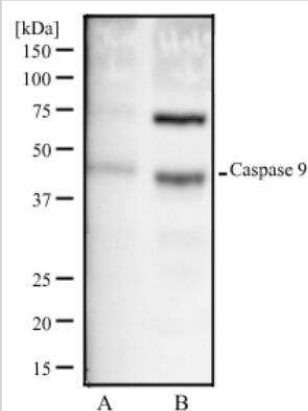
Product Information	
Unit Size	0.2 mg
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	LAP6 96-2-22
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein A or G purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	842
Gene Symbol	CASP9
Species	Human
Immunogen	Recombinant human Caspase 9 pro-domain protein. [UniProt# P55211]

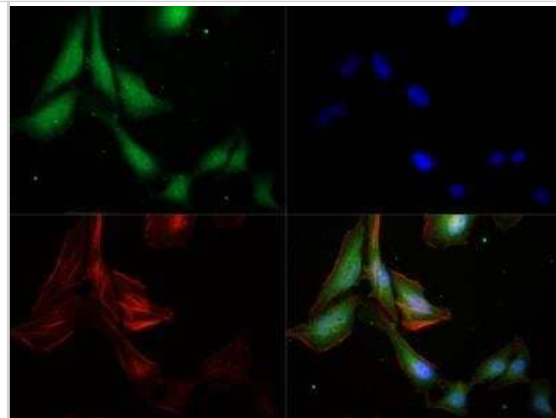
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockdown Validated
Recommended Dilutions	Western Blot 1 ug/ml, Simple Western 20 ug/ml, Immunohistochemistry 8-25 ug/ml. Use reported by customer review, Immunocytochemistry/ Immunofluorescence 2-10 ug/ml, Immunohistochemistry-Paraffin 8-25 ug/ml, Knockdown Validated
Application Notes	In Western Blot, bands at ~46kDa-48kDa and 35kDa are seen representing the pro and active forms of Caspase 9, respectively. In ICC/IF cytoplasmic staining can be seen in HeLa cells.

Images

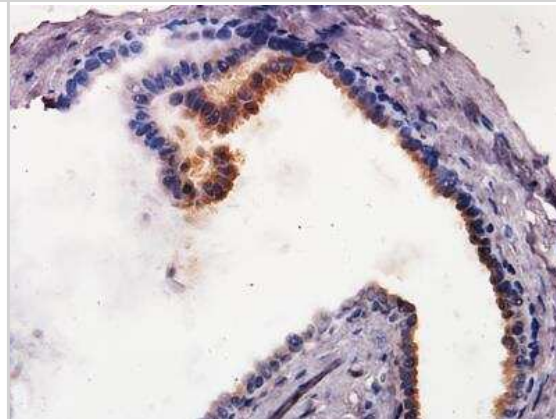
Western Blot: Caspase 9 Antibody (LAP6 96-2-22) [NB500-209] - Western blot analysis of Jurkat (A) and HeLa (B) cell lysate using Caspase 9 (NB500-209) antibody at 1:500.



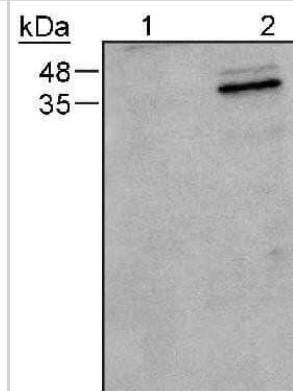
Immunocytochemistry/Immunofluorescence: Caspase 9 Antibody (LAP6 96-2-22) [NB500-209] - The Caspase 9 antibody was tested in HeLa cells against Dylight 488 (Green). Alpha-tubulin and nuclei were counterstained against Dylight 550 (Red) and DAPI (Blue).



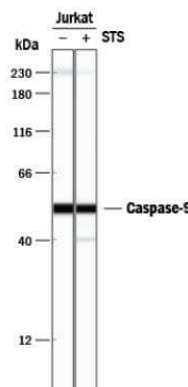
Immunohistochemistry-Paraffin: Caspase 9 Antibody (LAP6 96-2-22) [NB500-209] - IHC analysis of paraffin-embedded human breast carcinoma using Caspase 9 antibody at 1:25. Image from verified customer review.



Western Blot: Caspase 9 Antibody (LAP6 96-2-22) [NB500-209] - The pro and active forms of Caspase 9 detected in active 293 cell lysate (lane 2). Lane 1: inactive 293 cell lysate.



Simple Western: Caspase-9 Antibody (LAP6 96-2-22) [NB500-209] - Western lane view shows lysates of Jurkat human acute T cell leukemia cell line untreated (-) or treated (+) with 1 mM Staurosporine (STS) for 3 hours, loaded at 0.2 mg/mL. A specific band was detected for Caspase 9 at approximately 53 kDa (as indicated) using 20 ug/mL of Mouse Anti-Caspase 9 Monoclonal Antibody (Catalog # NB500-209). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



Publications

Kim H, Lee KH, Park IA et al. Expression of SIRT1 and apoptosis-related proteins is predictive for lymph node metastasis and disease-free survival in luminal A breast cancer. *Virchows Arch*. 2015-08-18 [PMID: 26280894] (IHC-P, Human)

Details:

Caspase 3 antibody was used at 1:300 dilution for IHC-P analysis of tissue sections from human cases of luminal A invasive breast ductal carcinoma. The assay was performed on benchmark automatic immunostaining device and the signal detection was performed using biotinylated anti-mouse secondary antibody -peroxidase-labeled streptavidin - DAB method.

Wu SH, Chyau CC, Chen JH et al. Anti-cancerous effects of *Wasabia japonica* extract in Hep3B liver cancer cells via ROS accumulation, DNA damage and p73-mediated apoptosis *Journal of Functional Foods* 2015-03-02 (WB, Human)

Hsuan SW, Chyau CC, Hung HY et al. The induction of apoptosis and autophagy by *Wasabia japonica* extract in colon cancer *Eur J Nutr* 2015-02-27 [PMID: 25720497] (WB, Human)

Martin, A et al. Apocytochrome c blocks caspase-9 activation and Bax-induced apoptosis. *J Biol Chem*;277(52):50834-41. 2002-12-27 [PMID: 12393884] (WB, Human)



Procedures

Western Blot Protocol for Caspase 9 Antibody (NB500-209)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Immunocytochemistry/Immunofluorescence Protocol for Caspase 9 Antibody (NB500-209)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.





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Products Related to NB500-209

NB800-PC6	293 Whole Cell Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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