Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

ANTI-CASPASE 9
Developed in Rabbit, Affinity Isolated Antibody

Product Number **C 7729**

Product Description

Anti-Caspase 9 is developed in rabbit using a synthetic peptide corresponding to amino acid residues 299-316 of human procaspase 9 with N-terminal added lysine conjugated to KLH with glutaraldehyde as immunogen. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-Caspase 9 specifically recognizes the proform and the active cleaved form of human caspase 9 by immunoblotting and immunoprecipitation (~47 kDa and ~35 kDa). An additional band of higher molecular weight (~70 kDa) may be detected in some cell line extracts. Staining of caspase 9 by immunoblotting is specifically inhibited with the immunizing peptide. The antibody is also useful for the detection of caspase 9 by immunohistochemistry. Cross-reactivity has been observed with human and rat.

Caspases are a family of intracellular proteases that mediate cell death and are the principal effectors of apoptosis. Caspase 9 (Mch6, ICE-LAP6, APAF3) is a member of the caspase 2 subfamily and is classified together with caspases 8 and 10 as an initiator caspase. It plays an important role in apoptosis induced by a variety of inducers such as TNFα, TRAIL, Anti-FAS, BAX, FADD, and TRADD. Caspase 9 functions as an initiator caspase when mitochondrial dysfunction is the primary event in apoptosis.[1-4]

The inactive procaspase 9 is a ubiquitous cytosolic protein. In some types of cells including cardiomyocytes and many neurons, it is localized in the intermembrane space of the mitochondria. When cytochrome c is released from the mitochondria into the cytosol, it forms a multimeric complex with the activating factor APAF1. This complex is then capable of recruiting procaspase 9 in a dATP/ATP dependent manner to form an apoptosome. Autocatalytic and transprocessing activation result in the formation of the mature active caspase 9 consisting of the prodomain and a large domain subunit as well as a ~10 kDa small subunit.

Following release of active caspase 9 from the apoptosome, it appears to accumulate in cell nuclei.[5] Caspase 9 can also be activated by granzyme B.[1]

Active caspase 9 can initiate a biochemical cascade involving cleavage and activation of the small-prodomain execution caspase -3, -6, and -7 with subsequent cleavage of various apoptotic substrates and culminating in the characteristic biochemical and morphological changes of apoptosis.[6]

Caspase 9 is essential for apoptosis during normal development of the murine central nervous system and plays a role in controlling tumor development.[7,8] Recently, direct regulation of caspase 9 by Akt kinase has been reported.[9] A short variant of caspase 9 lacking most of the large subunit including the catalytic domain was found to exhibit endogenous inhibition of apoptosis.[10]

Reagent

Anti-Caspase 9 is supplied as a solution
0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

Antibody concentration is approximately 1 mg/ml.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution.Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Procedure
Immunoprecipitation

Wash Buffer (RIPA Buffer) :
50 mM Tris Base, 0.25 % (w/v) Deoxycholate,
1 % IGEPAL, 150 mM NaCl, 1 mM EDTA, pH 7.4).
Alternatively: 20 mM HEPES buffer pH 7.5, containing 150 mM NaCl, 0.1 % (w/v) Triton X-100 and 10 % (w/v) glycerol was used for washing.

Preparation of Protein A

1. To 0.1 g of Protein A-Agarose (Product Number P 3391) add 0.8 ml deionized H_2O, allow swelling for 5 minutes.
2. Centrifuge 10 seconds at 12,000 x *g* at room temperature. Carefully discard supernatant.
3. Wash Protein A-Agarose with 1 ml washing buffer by resuspension.
4. Centrifuge for 10 seconds at 12,000 x *g* at room temperature. Discard supernatant. Repeat this step at least 2 more times.
5. Resuspend Protein A-Agarose in 0.4 ml washing buffer (50 % suspension) and divide it into aliquots of 50 to100 µl (approx. 25 to 50 µl agarose/bed volume).

Assay

1. To 50 to 100 µl of washed Protein A-Agarose suspension add 10 µg of Anti-Caspase 9 (Product Number C 7729).
2. Incubate for 15 minutes to 1 hour at room temperature, gently mixing the sample on a suitable shaker.
3. Centrifuge at 3,000 x *g* for 2 minutes at 4 °C. Discard supernatant.
4. Wash samples each with 1 ml washing buffer by resuspension and centrifugation at 3,000 x *g* for 2 minutes at 4 °C. Repeat this step at least 2 more times.
5. To each sample add 0.25 ml of mitochondria lysate. (500 µg HeLa mitochondria RIPA lysate.)
6. Incubate overnight at 4 °C, gently mixing the sample by end-over-end rocking.
7. Collect the immunoprecipitated complexes by centrifugation at 3,000 x *g* for 2 minutes at4 °C. Discard supernatant.
8. Wash each pellet with 1 ml washing buffer by resuspension and centrifugation at 3,000 x *g* for 2 minutes at 4 °C. Repeat this step at least 3 more times.
9. Resuspend each pellet in 75 µl Laemmli sample buffer to a final concentration of 1x sample buffer. Heat samples at 95 °C. for 5 minutes.
10. Spin down for 30 seconds at 12,000 x *g* at room temperature. Collect supernatant (IP sample).
11. Run samples and MW standards with known concentrations on minigel SDS-PAGE (appropriate percentage of polyacrylamide gel, according to the molecular size of the protein).
12. Transfer to nitrocellulose and perform immunoblotting.

Product Profile
A minimum working dilution of 1:300 is determined by immunoblotting using whole extract of Jurkat human acute T leukemia cells.

A minimum of 10 µg of the antibody immunoprecipitates caspase 9 from 500 µg of HeLa mitochondrial RIPA lysate.

A minimum working dilution of 1:40 is determined by indirect immunoperoxidase staining of microwave-treated, formalin-fixed, paraffin-embedded tissue sections of human and rat heart.

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

1. Duan, H., et al., J. Biol. Chem., **271,** 16720-16724 (1996).
2. Srinivasula, S.M., et al., J. Biol. Chem., **271,** 27099-27106 (1996).
3. Li, P., et al., Cell, **91,** 479-489 (1997).
4. Pan, G., et al., J. Biol. Chem., **273,** 5841-5845 (1998).
5. Krajewski, S.K., et al., Proc. Natl. Acad. Sci. USA **96**, 5752-5759 (1999).
6. Budihardjo, I., et al., Annu. Rev. Cell Dev. Biol., **15,** 269-290 (1999).
7. Kuida, K., Int. J. Biochem. Cell Biol., **32**, 121-124 (2000).
8. Soengas, M.S., et al., Science, **284,** 156-159 (1999).
9. Cardone, M.H., et al., Science, **282,** 1318-1321 (2000).
10. Seol, D.W., et al., J. Biol. Chem., **274,** 2072-2076 (1999).