

Product Information

Anti-Calpain-7 (Domain III, C-Terminal), Large Subunit

Developed in Rabbit
Affinity Isolated Antibody

Product Number **C 1864**

Product Description

Anti-Calpain-7 (Domain III, C-Terminal), Large Subunit is developed in rabbit using a synthetic peptide corresponding to the carboxyterminal end of domain III of the large subunit of human calpain 7 (capn-7, PalBH) as immunogen. The antibody is affinity purified using agarose to which the immunogen peptide has been bound.

Anti-Calpain-7 (Domain III, C-Terminal), Large Subunit recognizes human, rat, and mouse calpain 7 by immunoblotting. It recognizes the latent and amino-processed protein and does not crossreact with other calpain family members (calpain 1, calpain 2, calpain 3, LP-82/85 calpain, nCL-2, nCL-3, etc.). The antibody binds to the reduced protein. By immunoblotting against the reduced protein, the antibody reacts with bands at 93 kDa, 68 kDa, 48 kDa, and a series of smaller forms. Cleavage of the carboxyterminal region generates smaller forms of calpain 7, but it is not clear if these forms are proteolytically active.

Calpains are calcium-activated, non-lysosomal cysteine proteases that cleave cytoskeletal and submembranous proteins. The calpains have papain-like activity, thus the -pain nomenclature. The calpain (calcium-dependent proteinase or calcium activated neutral protease) system consists of two ubiquitous forms of calpain (calpain 1 and calpain 2), a series of tissue specific calpains (calpains 3-15), and a calpain inhibitory protein (calpastatin). The calpain system plays a regulatory role in cellular protein metabolism.¹ This regulatory role may have important implications in platelet aggregation and pathologies associated with altered calcium homeostasis and protein metabolism such as ischemic cell injury and degenerative diseases. Inhibitors of calpain have been shown to block dexamethasone- and low-level irradiation-induced apoptosis in thymocytes suggesting that calpain has a regulatory or mechanistic role in apoptotic cell death.

The "classical" calpain family members (calpain 1 and calpain 2) are heterodimers and consist of a common regulatory small subunit (calpain-S1), and a large variable catalytic subunit. Domains in the large subunit

include the amino-terminal domain I, the proteinase domain II,² domain III, and EF-hand (Ca²⁺-binding) domain-IV.¹ Calpain 7, also known as PalBH (the human orthologue of the *A. nidulans* Pal-B protein), is an intracellular cysteine protease. In *Aspergillus*, calpain 7 cleaves the transcription factor PacC, and is involved in pH signaling.^{2,3} Calpain 7, (like calpain 5 and calpain 6) lacks the EF-Hand calcium binding domains of the "classical" calpains, instead having a second, modified domain-III (called domain N). Since calpain 7 lacks domain-IV, it is unclear if calcium affects its activity.^{2,4} Also, unclear is any similarity in function of calpain 7 in mammals and to the utility in *Aspergillus*. It is not known if autolytic cleavage of the propeptide region (as in calpain 1 and calpain 2) occurs with dissociation of the small subunit and membrane binding, and if calpain 7 associates with a small subunit.⁵ The latent large subunit is 93 kDa, and the aminoterminal truncations at activation yields approximately 68 kDa isoforms. Also, a cascade of smaller forms truncated at the N-terminus and C-terminus can be seen with further activation.

Calpain 7, like calpain 1 and calpain 2, is ubiquitously expressed. Calpains are present in all mammalian tissues and are involved in a variety of processes including cytoskeletal reorganization, muscle protein degradation,¹ cell proliferation,^{6,7} differentiation,⁸⁻¹⁰ and vesicular secretion.

Calpastatin, the endogenous inhibitor of calpain-1 and calpain 2, is also ubiquitously expressed, in molar excess compared to the enzymes. Many different splice variants occur in calpastatins, which may lead to different inhibition profiles for the different calpains.⁹ It is not clear if calpastatin inhibits calpain 7.

Mutations in calpains have been linked to diseases such as muscular dystrophy and type II diabetes, and calpains also appear to play a role in the caspase system of apoptosis.^{11,12}

Reagent

Anti-Calpain-7 (Domain III, C-Terminal), Large Subunit is supplied as approximately 1 mg/ml of antibody in 0.01 M phosphate buffered saline containing 50% glycerol and 0.05% sodium azide.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, the solution may be stored at 0 °C to -20 °C. Do not store in a frost-free freezer. The antibody is supplied with 50% glycerol to prevent freezing. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data 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.

Product Profile

For immunoblotting, a working antibody dilution of 1:1,000 is recommended using an alkaline phosphatase conjugated secondary antibody and a colorimetric substrate such as BCIP/NBT. For chemiluminescent substrates, a working antibody dilution of 1:5,000 is recommended.

Note: Higher concentrations of antibody may be needed for samples from more distantly related species. Since calpain 7 is a cellular protein, cell lysates work well for immunoblotting. EDTA/EGTA treatment of tissues or lysates may be required to detect the latent zymogen.

In order to obtain the best results using various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

1. Johnson, G.V., and Guttman, R.P., Calpains: intact and active? *Bioessays*, **19**, 1011-1018 (1997).
2. Denison, S.H., et al., Signaling of ambient pH in *Aspergillus* involves a cysteine protease. *J. Biol. Chem.*, **270**, 28519-28522 (1995).
3. Diez, E., et al., Activation of the *Aspergillus* PacC zinc finger transcription factor requires two proteolytic steps. *EMBO J.*, **21**, 1350-1359 (2002).
4. Dear, N., et al., A new subfamily of vertebrate calpains lacking a calmodulin-like domain: Implications for calpain regulation and evolution. *Genomics*, **45**, 175-184 (1997).
5. Franz, T., et al., Capn7: A highly divergent vertebrate calpain with a novel C-terminal domain. *Mammalian Genome*, **10**, 318-321 (1999).
6. Ariyoshim, H., et al., Possible involvement of m-calpain in vascular smooth muscle cell proliferation. *Arterioscler. Thromb. Vasc. Biol.*, **18**, 493-498 (1998).
7. Kulkarni, S., et al., Calpain mediates integrin-induced signaling at a point upstream of Rho family members. *J. Biol. Chem.*, **274**, 21265-21275 (1999).
8. Balcerzak, D., et al., An antisense oligodeoxynucleotide to m-calpain mRNA inhibits myoblast fusion. *J. Cell Sci.*, **108**, 2077-2082 (1995).
9. Murray, S.S., et al., The calpain-calpastatin system and cellular proliferation and differentiation in rodent osteoblastic cells. *Exp. Cell Res.*, **233**, 297-309 (1997).
10. Stockholm, D., et al., Studies on calpain expression during differentiation of rat satellite cells in primary cultures in the presence of heparin or a mimic compound. *Exp. Cell Res.*, **252**, 392-400 (1999).
11. Zongchao, J., et al., Mutations in calpain-3 associated with limb girdle muscular dystrophy: analysis by molecular cloning and mutation in M-calpain. *Biophys. J.*, **80**, 2590-2596 (2001).
12. Horikawa, Y., et al., Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nature Genetics*, **26**, 163-175 (2000).

KAA/JPA 07/03

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.