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ProductInformation

Anti-Calpain-5 (Domain IV, C-Terminal), Large Subunit
Developed in Rabbit
Affinity Isolated Antibody

Product Number **C 1114**

Product Description

Anti-Calpain-5 (Domain IV, C-Terminal), Large Subunit is developed in rabbit using a synthetic peptide corresponding to the carboxyterminal end of domain IV in the large subunit of human calpain-5 (capn-5, hTra-3) as immunogen. The antibody is affinity purified using agarose to which the immunogen peptide has been bound.

Anti-Calpain-5 (Domain IV, C-Terminal), Large Subunit detects human, rat, and mouse calpain-5 by various immunochemical techniques including immunoblotting, immunoprecipitation, immunohistochemistry, and ELISA. The antibody recognizes the latent and the active protein. It binds calpain-5 and does not cross-react 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 and non-reduced protein. By immunoblotting against the reduced protein, the antibody reacts with bands at 73 kDa, 58 kDa, and a series of smaller forms.

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 dexa-methasone- 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 aminoterminal domain-I, the proteinase domain-II,[2] domain-III, and EF-hand (Ca^{2+}-binding) domain-IV.[1] Calpain-5, also known as hTra-3 (the human orthologue of the *C. elegans* sex determination gene), is an intracellular cysteine protease.[3] Calpiain-5 lacks the EF-Hand calcium binding domains of the “classical” calpains, and it is unclear if calcium effects the activity.[4] It is also unclear if the function of calpain-5 in mammals bears any similarity to the utility in *C. elegans* and if autolytic cleavage of the propeptide region (as in calpain-1 and calpain-2) occurs with dissociation of the small subunit and membrane binding. In fact, it is unclear if calpain-5 associates with a small subunit. The latent large subunit is 73 kDa, and the amino-terminal truncations at activation yield approximately a 58 kDa isoform. Also, a cascade of smaller forms truncated at the aminoterminal and carboxyterminal ends can be seen with further activation.

Calpain-5, unlike calpain-1 and calpain-2, is not 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,[5, 6] differentiation,[7-9] 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 various calpains.[8] It is not clear if calpastatin inhibits calpain-5.

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.[10, 11]

Reagent
Anti-Calpain-5 (Domain IV, C-Terminal), Large Subunit is supplied as 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 concentration 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 concentration of 1:5,000 is recommended.

For ELISA, immunoprecipitation, and immunohistochemistry, we recommend determining working dilutions by titration.

Note: Higher concentrations of antibody may be needed for samples from more distantly related species. Since calpain-5 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 preparation, we recommend determining the optimal working dilution by titration.

References

1. Johnson, G.V., and Guttmann, R.P., Calpains: intact and active? Bioessays, **19**, 1011-1018 (1997).
2. Matena, K., et al., Genomic organization of mouse *Capn5* and *Capn6* genes confirms that they are a distinct calpain subfamily. Genomics, **48,**117-120 (1998).
3. 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).
4. Kuwabra, P.E., and Perry, M.D., It ain't over till it's ova: germline sex determination in *C. elegans*. Bioessays, **23**, 596-604 (2001).
5. Ariyoshim, H., et al., Possible involvement of m-calpain in vascular smooth muscle cell proliferation. Arterioscher. Thromb. Vasc. Biol., **18**, 493-498 (1998).
6. Kulkarni, S., et al., Calpain mediates integrin-induced signaling at a point upstream of Rho family members. J. Biol. Chem., **274**, 21265-21275 (1999).
7. Balcerzak, D., et al., An antisense oligodeoxy-ribonecleotide to m-calpain mRNA inhibits myoblast fusion. J. Cell Sci., **108**, 2077-2082 (1995).
8. 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).
9. 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).
10. 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).
11. 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).

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