

## Product Information

### Anti-Calpain-5 (Domain I, N-Terminal), Large Subunit

Developed in Rabbit  
Affinity Isolated Antibody

Product Number **C 0989**

#### Product Description

Anti-Calpain-5 (Domain I, N-Terminal), Large Subunit is developed in rabbit using a synthetic peptide corresponding to the aminoterminal end of domain I of 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 I, N-Terminal), Large Subunit recognizes the first 30 amino acids of the "propeptide domain" (domain I) of calpain-5, aminoterminal from the activation cleavage site. The antibody binds to human, mouse, and rat calpain-5, and does not crossreact with other calpain family members (calpain-1, calpain-2, calpain-3, LP-82/85 calpain, nCL-2, nCL-3, etc.). It can be used in various immunochemical techniques including immunoblotting, immunoprecipitation, immunohistochemistry, and ELISA. The antibody binds to the reduced and non-reduced protein. By immunoblotting against the reduced protein, the antibody reacts with a band at 73 kDa, 58 kDa, 48 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.<sup>1</sup> 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,<sup>2</sup> domain-III, and EF-hand (Ca<sup>2+</sup>-binding) domain-IV.<sup>1</sup> Calpain-5, also known as hTra-3 (the human orthologue of the *C. elegans* sex determination gene), is an intracellular cysteine protease.<sup>3</sup> Calpain-5 lacks the EF-Hand calcium binding domains of the "classical" calpains, and it is unclear if calcium effects the activity.<sup>4</sup> It is also unclear if the function of calpain-5 in mammals bears any similarity to the utility in *C. elegans* and whether 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,<sup>1</sup> cell proliferation,<sup>5,6</sup> differentiation,<sup>7-9</sup> 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.<sup>8</sup> 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.<sup>10,11</sup>

## Reagent

Anti-Calpain-5 (Domain I, N-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 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.

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 preparations, we recommend determining the optimal working dilution by titration.

## References

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