

## Product Information

### **ANTI-CALPAIN-94 (DOMAIN III, INSERT 2), LARGE SUBUNIT** Developed in Rabbit, Affinity Isolated Antibody

Product Number **C 6236**

#### **Product Description**

Anti-Calpain-94 (Domain III, Insert 2), Large Subunit is developed in rabbit using a synthetic peptide corresponding to insert 2, in domain-III of the large subunit of human calpain-94 (calpain-3, nCANP, nCL-1, calpain p94, muscle calpain) as immunogen. The antibody is affinity purified using agarose to which the immunogen peptide has been bound.

This antibody recognizes insert 2 of domain III in the large subunit of human calpain-94 by various immunochemical techniques including immunoblotting, immunoprecipitation, immunohistochemistry, and ELISA. The antibody recognizes both the latent and active protein. It binds to human calpain-94, but does not crossreact with other calpain family members ( $\mu$ -calpain, m-calpain, 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 94 kDa, 82 kDa, 62 kDa, 60 kDa, and a series of smaller active 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 (m-calpain and  $\mu$ -calpain), a tissue specific calpain (n-calpain), 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 calpain family members are heterodimers and consist of a common small subunit (regulatory), and a large variable subunit (catalytic). Domains in the large subunit include the amino-terminal domain-I, the proteinase domain-II, domain-III, and EF-hand ( $\text{Ca}^{2+}$ -binding) domain-IV.<sup>1</sup> Calpain-94, also known as calpain-3 and muscle calpain, is an intracellular, calcium-dependent cysteine protease. The latent large subunit is 94 kDa, and the amino-terminal truncation at activation yields approximately 64-62 kDa isoforms. Also, a cascade of smaller forms can be seen with further activation. Unlike m-calpain and  $\mu$ -calpain, which are ubiquitously expressed, calpain-94 is a tissue-specific calpain. The half-life of native calpain-94 is much shorter (approximately 10 minutes) than the other calpains. The endogenous calpain inhibitor, calpastatin, does not inhibit calpain-94 as it does the other calpains. Mutations in calpain-94 have been linked to Limb-Girdle Muscular Dystrophy.

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>2,3</sup> differentiation,<sup>4-6</sup> and vesicular secretion.

#### **Reagent**

Anti-Calpain-94 (Domain III, Insert 2), 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 concentrations by titration.

Note: Higher concentrations of antibody may be needed for samples from more distantly related species. Since calpain-94 is a cellular protein, cell lysates work well for immunoblotting. EDTA/EGTA treatment of tissues or lysates is required to detect the latent zymogen.

In order to obtain the best results using different techniques and preparations we recommend determining the optimal working concentrations by titration test.

### References

1. Johnson, G.V., and Guttman, R.P., Calpains: intact and active? *Bioessays*, **19**, 1011-1018 (1997).
2. Ariyoshim, H., et al., Possible involvement of m-calpain in vascular smooth muscle cell proliferation. *Arterioscler. Thromb. Vasc. Biol.*, **18**, 493-498 (1998).
3. Kulkarni, S., et al., Calpain mediates integrin-induced signaling at a point upstream of Rho family members. *J. Biol. Chem.*, **274**, 21265-21275 (1999).
4. Balcerzak, D., et al., An antisense oligodeoxyribonucleotide to m-calpain mRNA inhibits myoblast fusion. *J. Cell Sci.*, **108**, 2077-2082 (1995).
5. 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).
6. 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).

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