# Novocastra™ Lyophilized Mouse Monoclonal Antibody Calpain

BIOSYSTEMS

## Product Code: NCL-CALP-2C4

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| **Intended Use** | FOR RESEARCH USE ONLY. |
| **Specificity** | This antibody reacts with full-size calpain 3 (94 kD) plus an additional fragment at 30 kD in human skeletal muscle. A band of 94 kD is seen with rabbit and dog muscle while extracts of hamster muscle show reactivity with the 94 kD band and a larger species of approximately 110 kD. This 110 kD band is the principal immunoreactive species seen in rat muscle extracts. This antibody produces no bands with mouse, pig or chicken muscle. |
| **Clone** | Calp3d/2C4 |
| **Ig Class** | IgG2b |
| **Antigen Used for Immunizations** | Synthetic peptide containing amino acids 1–19 of the human calpain 3 sequence. |
| **Hybridoma Partner** | Mouse myeloma (X63.Ag8.653). |
| **Preparation** | Lyophilized tissue culture supernatant containing 15 mM sodium azide.<br>Reconstitute with the volume of sterile distilled water indicated on the vial label. |
| **Effective on Frozen Tissue** | No |
| **Effective on Paraffin Wax Embedded Tissue** | No |
| **Recommendations on Use** | Western Blotting: Typical working dilution 1:25–1:50. The optimal dilution should be determined by the user as calpain must be homogenized in treatment buffer (see Positive Controls below) immediately after harvest to prevent degradation. |
| **Positive Controls** | Western Blotting: Freshly isolated rat or normal human skeletal muscle homogenate prepared in treatment buffer containing 0.125 M tris-HCl buffer pH 6.4, 10% glycerol, 4% SDS, 4 M urea, 10% mercaptoethanol and 0.001% bromophenol blue (final pH of treatment buffer, 6.8). |
| **Staining Pattern** | A band at 110 kD in rat muscle extract and/or bands at approximately 94 and 30 kD in Western blots of human muscle extracts. |
| **Storage and Stability** | Store unopened lyophilized antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. The reconstituted antibody is stable for at least two months when stored at 4 °C. For long term storage, it is recommended that aliquots of the antibody are frozen at -20 °C (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use. |
| **General Overview** | Muscle specific calcium activated neutral protease, or calpain-3, is a 94 kD nonlysosomal intracellular cysteine protease. It belongs to the calpain family, which includes three ubiquitous enzymes and several tissue specific forms. The ubiquitous calpains are dimers composed of large catalytic subunits of approximately 80 kD and a common 30 kD regulatory subunit. Tissue specific enzymes, such as calpain-3, appear to lack the regulatory subunit. The large subunits of calpains can be divided into four domains. The N-terminal region of domain I is autocatalytically cleaved during activation by calcium ions and it has been suggested that domain I may be involved in the regulation of proteolytic activity. Domain II is thought to contain the cysteine proteases active site, no function has been assigned to domain III and domain IV contains structures involved in calcium binding. In addition calpain-3 has three unique regions, namely NS at the beginning of domain I, IS1 in the protease domain and IS2 in domain III. These regions show little homology to other proteins but IS2 contains a nuclear translocation signal. It is thought that these regions may be important for the muscle specific function of calpain-3. Originally, calpain 3 was thought to be undetectable in skeletal muscle due to very rapid autolysis, however, studies with the Novocastra monoclonal antibodies have demonstrated that calpain 3 protein is stable and detectable in human muscle, when homogenized in treatment buffer immediately after harvest. |
| **General References** | Anderson L V B, Davison K, Moss J A, et al.. American Journal of Pathology. 153 (4): 1169–1179 (1998).<br>Anderson L V B. Multiplex Western blot analysis of the muscular dystrophy proteins. In Bushby K M D and Anderson L V B (eds) “Muscular Dystropy: Methods and Protocols”, Methods in Molecular Medicine series.<br>Topaloglu H, Dinçer P, Richard I, et al.. Neuropediatrics. 28: 212–216 (1997). |

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