

## Product Information

### ADAM-10, mouse recombinant, expressed in Sf21 cells

Catalog Number **A9975**  
Storage Temperature  $-20\text{ }^{\circ}\text{C}$

Synonyms: Kuzbanian, mammalian disintegrin metalloprotease, or myelin-associated metalloproteinase

#### Product Description

Recombinant Mouse ADAM-10 is produced from the DNA sequence encoding the human CD33 signal peptide and the ectodomain of recombinant mouse ADAM-10 (amino acid residues 19-673).<sup>1</sup> It is expressed with a C-terminal histidine tag in Sf 21 insect cells. Purified recombinant mouse ADAM-10, predicted molecular mass of ~52 kDa and 469 amino acids, exists as the mature and active form with the N-terminal sequence of T<sub>215</sub>TLAERNTCQ. By immunoblotting, the apparent molecular mass is ~60 kDa.

ADAM-10 is a member of the ADAM (a disintegrin and metalloprotease-like domain) family.<sup>1,2</sup> It contains a pro domain with a cysteine switch and furin cleavage sequence, catalytic domain with the zinc-binding site and Met-turn (expected for reprotolysins), disintegrin-like domain, cystein-rich domain, EGF-like domain, transmembrane domain, and cytoplasmic domain. ADAM-10 is highly conserved. Mouse, rat, cattle, and human ADAM-10 share 97% amino acid identity. There is 45% identity between mouse and *Drosophila* ADAM-10.

ADAM-10 is widely expressed in tissues and can be found on the cell surface and within the cell.<sup>3</sup> Active ADAM-10 has an important role in neurogenesis (processing notch, notch ligand delta, and amyloid protein precursor at the alpha site).<sup>4</sup> ADAM-10 also cleaves myelin basic protein and type IV collagen.<sup>5</sup>

Recombinant Mouse ADAM-10 is lyophilized from a 0.2  $\mu\text{M}$  filtered solution in 25 mM MES, 0.2 M sodium chloride, 5 mM calcium chloride, 2.5 mM zinc chloride, 0.005% Brij<sup>®</sup> L23, pH 5.0.

Purity: >90% (SDS-PAGE, visualized by silver stain)

Endotoxin level:  $\leq 1\text{ EU}/\mu\text{g}$   
(LAL [Limulus ameocyte lysate] method)

Recombinant Mouse ADAM-10 cleaves the coumarin peptide substrate, (7-methoxycoumarin-4-yl) acetyl-Pro-Leu-Ala-Gln-Ala-Val-(3-[2, 4-dinitrophenyl]-L-2,3-diaminopropionyl)-Arg-Ser-Ser-Ser-Arg-NH<sub>2</sub>, and is measured by fluorescence (excitation at 320 nm and emission at 405 nm). The activity is measured with 10  $\mu\text{M}$  coumarin substrate and 100 ng of enzyme (ADAM-10) in a total of 100  $\mu\text{l}$  reaction mixture (50 mM HEPES, 5  $\mu\text{M}$  zinc chloride, 0.01% Brij L23, pH 7.5) at 37  $^{\circ}\text{C}$ . Under these conditions the specific activity is >5 pmoles/min/ $\mu\text{g}$ . In order to achieve the optimal activity, it is strongly recommended to use the described assay conditions. Use of other conditions may result in lower activity.

Recombinant Mouse ADAM-10 releases TNF $\alpha$  from a fusion protein containing proTNF $\alpha$ .<sup>6</sup> It also cleaves the oxidized insulin B-chain. The major cleavage sites are Ala<sup>14</sup>-Leu<sup>15</sup> and Tyr<sup>16</sup>-Leu<sup>17</sup>.

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

#### Preparation Instructions

Reconstitute the contents of the vial with 25 mM Tris, pH 7.5, to give the desired stock solution (0.1 mg/ml recommended).

#### Storage/Stability

Store the product at  $-20\text{ }^{\circ}\text{C}$ . ADAM-10 remains active for greater than six months at  $-20\text{ }^{\circ}\text{C}$  to  $-70\text{ }^{\circ}\text{C}$ .

Upon reconstitution, this enzyme can be aliquoted and stored at  $-20\text{ }^{\circ}\text{C}$  for up to one month and  $-70\text{ }^{\circ}\text{C}$  for three months without detectable loss of activity. Repeated freezing and thawing is not recommended. Do not store in a "frost-free" freezer.

## References

1. Pan, D., and Rubin, G.M., *Cell*, **90**, 271-280 (1997).
2. Rooke, J., *et al.*, *Science*, **273**, 1227-1231 (1996).
3. Fahrenholz, F., *et al.*, *Ann. N.Y. Acad. Sci.*, **920**, 215-222 (2000).
4. Lammich, S., *et al.*, *Proc. Natl. Acad. Sci. USA*, **96**, 3922-3927 (1999).
5. Millichip, M.I., *et al.*, *Biochem. Biophys. Res. Comm.*, **245**, 594-598 (1998).
6. Rosendahl, M.S., *et al.*, *J. Biol. Chem.*, **272**, 24588-24593 (1997).

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JR,RC,CS,KAA,MAM 07/12-1