

Cathepsin K proenzyme (human, recombinant)
(EC 3.4.22.38)

CATALOG NO.: SE-367

LOT NO.: Temp

DESCRIPTION: MW 35.3 kDa calculated; ~38 kDa by SDS-PAGE. Recombinant glycosylated procathepsin K (also known as cathepsin O or cathepsin O2)¹ cloned from human cDNA, expressed in insect cells, and purified as full-length proenzyme. Cathepsin K, a member of the papain family of cysteine proteases, acts upon proteins such as collagen, AL amyloid, kinin, and elastin²⁻⁵. It is a lysosomal protease expressed primarily in osteoclasts, but also in other cell types such as macrophages^{6,7}. It functions in bone remodeling and is implicated in disease states such as atherosclerosis, arthritis, and pycnodysostosis^{5,8,9}.

PURITY: >95% by SDS-PAGE.

USAGE: Study enzyme kinetics, cleave target substrates, and screen for inhibitors⁹. To activate proenzyme, add 32.5 mM NaOAc pH 3.5 (prepared fresh) to 20% of procathepsin K volume (e.g., 5 μ l procathepsin K + 1 μ l 32.5 mM NaOAc pH 3.5), and incubate at room temperature for 2-3 hours. **Note:** this is meant only as a guideline, based on in-house activation of each lot (see figure below). Because many factors can affect speed of activation, if optimal activation is desired the user *must* determine the exact conditions empirically. **Caution:** once procathepsin K is activated, it will rapidly autodegrade, even at -80°C. The following methods will delay autodegradation: 1) Adjust pH to 8.0 (and dilute if possible), and immediately freeze at -80°C. 2) Lyophilize; 3) Add MMTS (methylmethane thiosulfonate) or HgCl₂ to 100 μ M and freeze at -80°C. To restore MMTS-inhibited activity, add DTT to 2 mM or L-cysteine to 3 mM¹⁰⁻¹². Assay cathepsin K with substrates Z-Phe-Arg-AMC (Cat. # P-139), Z-Phe-Arg-pNA (Cat. # P-140), Z-Gly-Pro-Arg-AMC (Cat. # P-142), or Z-Leu-Arg-AMC (Cat. # P-229) in 150 mM NaAcetate pH 5.5, 20 mM cysteine, 5 mM EDTA, 4% DMSO. **Important:** Briefly centrifuge vial prior to opening.

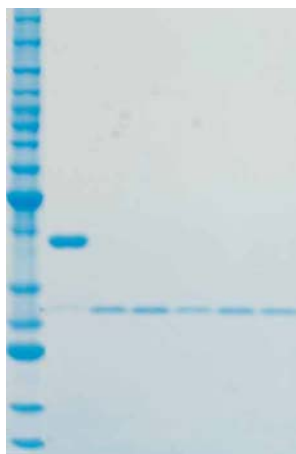


Figure: Activation of procathepsin K at 22°C. Lane 1, size marker. Lane 2, 0 hours. Lane 3, 3 hours. Lane 4, 4 hours. Lane 5, 5 hours. Lane 6, 6 hours. Lane 7, 7 hours. 1.5ug per lane, loaded after EtOH precipitation/wash and resuspension in SDS-PAGE sample buffer.

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SUPPLIED AS: 10 µg protein at 0.687 µg/µl in 14.6 µl 20 mM sodium phosphate, pH 6.9, 500 mM NaCl, 1 mM EDTA, 30% glycerol.

STORAGE: -80°C. After initial defrost, aliquot product into individual tubes and refreeze at -80°C. Avoid repeated freeze/defrost cycles and extended periods unfrozen.

Note: This enzyme is stable when stored as received under the above conditions. Procedures such as dilution of the enzyme followed by refreezing could lead to loss of activity.

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