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Recombinant Human HtrA1 His-tagged

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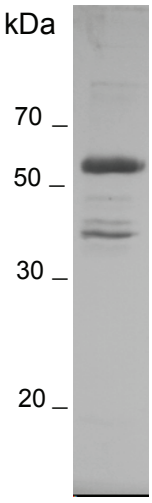
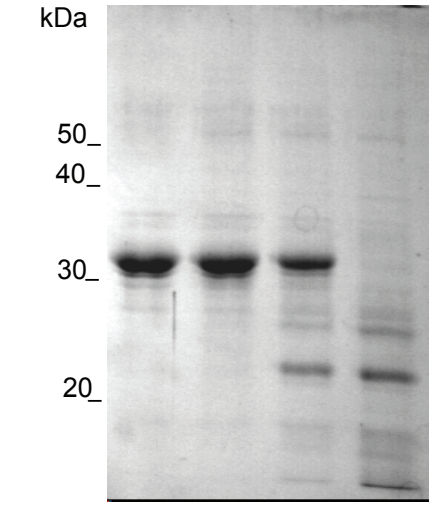
INTRODUCTION

Secreted human HtrA1 serine protease is supposed to form oligomers of identical protein chains as revealed by X-ray analysis for the bacterial HtrA protein DegP [1]. Polypeptide chains of human HtrA1 consist of several domains: An N-terminal insulin-like growth factor domain is followed by a Kazal-type serine protease inhibitor domain, a linker region, a trypsin-like protease domain and a PDZ domain [2]. The function of HtrA1 appears closely linked to signaling by proteins of the TGFß family [3]. During mouse embryo development, HtrA1 is localized in specific areas where signaling by TGFß family proteins occurs. HtrA1 binds TGFß, BMP4, Gdf5 and activin. It inhibits signaling by these factors. For inhibition serine protease activity of HtrA1 is essential [3]. HtrA1 is upregulated in osteoarthritic cartilage [4]. Increased cartilage HtrA1 may possibly aggravate osteoarthritis by antagonizing TGFß and thereby promoting terminal differentiation of articular chondrocytes. In later stages of tumor progression, HtrA1 is downregulated [5, 6]. As a protease, HtrA1 hydrolyzes type II procollagen α1 C-propeptide [7], decorin and biglycan [3]. Proteolytic activity of HtrA1 is regulated by ligand binding to the PDZ domain [7].



SDS-PAGE of 2 µg HrtA1 protein



Hydrolysis of ß-Casein by HtrA1

ß-Casein (0.5 mg/ml) was incubated in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM CaCl2 without or with HtrA1 (5 µg/ml). After various time intervals aliquots of the reaction mixtures were withdrawn and analyzed by SDS-PAGE.

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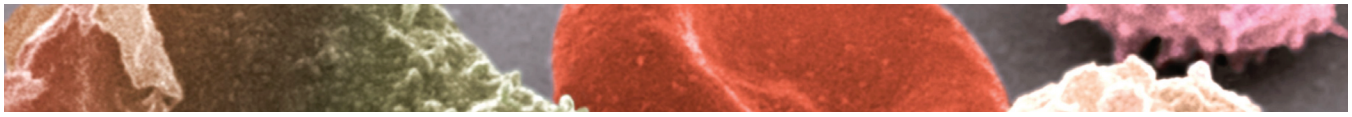

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MOLECULAR FORM

Recombinant human HtrA1 is expressed in insect cells with a C-terminal His-tag and purified from insect cell culture supernatants. The calculated Mr of secreted HtrA1 is 50 kDa. HtrA1 is solublized in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM CaCl2, 0.05 % Brij-35.

PURITY

Recombinant HtrA1 appears as a major protein of about 53 kD in SDS-PAGE (> 80 % of total protein). Minor bands of HtrA1 fragments may be visible in the enzyme preparation.

ENZYMATIC ACTIVITY

Proteolytic activity of recombinant human HtrA1 is documented by digestion of ß-casein. 0.5 mg ß-casein/ml are completely digested by 5 µg/mL HtrA1 within 3 hours at 37 ºC (see figure above).

STABILITY & STORAGE

Recombinant human HtrA1 is stable until the expiry date given on the label when stored at -20 °C. Repeated freezing and thawing should be avoided.

APPLICATIONS

Recombinant HtrA1 allows detailed studies of the structure and function of this protease. The enzyme is used to screen for inhibitors and to characterise inhibitor actions. Recombinant HtrA1 can also serve as standard in enzymatic and immunochemical assays.

REFERENCES

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