

Note that this data sheet is not lot-specific. Please consult the vial label and the certificate of analysis for information on specific lots.

Recombinant Aggrecan IGD (Interglobular Domain T331 - G458)

Catalogue number: 30 411 004

Package size: 100 µg / 200 µl

1. Protein characteristics

1.1 Molecular form: The polypeptide connecting human aggrecan globular domains 1 and 2 (T₃₃₁ - G₄₅₈) is expressed in *E. coli* with a C-terminal His-tag. The recombinant protein contains cleavage sites for aggrecanases (E₃₇₃ - A₃₇₄ in human aggrecan) and matrix metalloproteinases (N₃₄₁ - F₃₄₂ in human aggrecan). It comprises the following amino acids:

TAEDFVDIPENFFGVGG EEDITVQTVTWPDMELPLPRNITEGEARGSVILTVKPIFEVSPSPLEPE
EPFTFAPEIGATAFAEVENETGEATRPWGFPTPGLGPATAFTSEDLVVQVTA VPGQPHLP GG
(His-tag)

Main cleavage sites are indicated by arrows. The calculated M_r of the His-tagged protein is 15 493 Da. The protein is solubilized in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM CaCl₂.

1.2 Purity: The recombinant aggrecan interglobular domain appears as a major band at about 21 kDa in SDS-PAGE. It represents more than 80 % of total protein in the preparation (Fig. 1).

1.3 Stability and storage: Aggrecan interglobular domain is stable until the expiry date given on the label when stored at -70°C. The protein can be kept at -20°C for several weeks and on ice for several days. Repeated freezing and thawing should be avoided.

2. Applications

Aggrecan interglobular domain is used as substrate for aggrecanases and matrix metalloproteinases. For proteinase activity measurements, the protein is incubated with proteinase for various time intervals. Thereafter, aliquots of the incubation mixture are analyzed by SDS-PAGE or by ELISA.

Upon cleavage with aggrecanases the apparent M_r of aggrecan interglobular domain in SDS-PAGE is reduced from 21 kDa to about 13 kDa (Fig. 1). Quantitative measurement of aggrecanase cleavage requires a neoepitop antibody with specificity for the N-terminus A R G S V I L T . . . appearing upon hydrolysis. The fragment with the newly formed N-terminus is fixed by the neoepitop antibody to a microplate and quantified with an anti-His-tag antibody. In analogy, cleavage by matrix metalloproteinases can be measured with antibodies to neoepitopes appearing upon action of these enzymes.

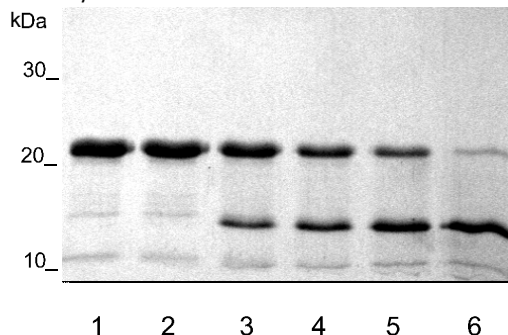


Fig. 1 Hydrolysis of recombinant aggrecan interglobular domain with isolated ADAMTS4

Recombinant aggrecan-IGD1 at a concentration of 10 µM in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM CaCl₂ was incubated without and with 0.1 µM truncated ADAMTS4 (ADAMTS4, Δ580-837) for various time intervals. Aliquots with 1.5 µg aggrecan-IGD1 were then analyzed by SDS-PAGE. Lane 1: Aggrecan-IGD1 incubated without ADAMTS4, Lanes 2-6: Digestion mixtures of aggrecan-IGD1 with ADAMTS4 after 0, 0.5, 1, 2 and 5 hours incubation.

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3. Introduction to aggrecan structure and degradation

Aggrecan is a large aggregating proteoglycan of articular cartilage. It is also found in aorta, discs and tendons [1, 2]. The aggrecan core protein consists of 2317 amino acids [2]. Up to 130 glucosamino-glycan chains are attached to the core protein and the total molecular mass can reach $2.2 - 3.0 \times 10^6$ Daltons [4].

Within the aggrecan molecule 3 globular domains G1, G2 and G3 can be distinguished. Domains G1 and G2 are connected by a rod-shaped polypeptide called interglobular domain (IGD), while the sequence between domains G2 and G3 contains attachment regions for keratan sulfate and chondroitin sulfate chains. Aggrecan interacts via the G1 domain with hyaluronan and link protein to form large aggregates. Such aggregates can contain up to 50 - 100 aggrecan monomers noncovalently bound to a single hyaluronan chain through 2 link proteins [1, 2, 4]. The aggregates form a hydrated gel-like structure, which endows cartilage with resistibility to compression and deformation.

Degradation of aggrecan appears to initiate at the C-terminus. The population of aggrecan molecules without the G3 domain increases with ageing [5]. Isolated aggrecanases cleave aggrecan at 4 sites within the chondroitin sulfate -rich region (sites E₁₆₆₇ - G₁₆₆₈, E₁₄₈₀ - G₁₄₈₁, E₁₇₇₁ - A₁₇₇₂, E₁₈₇₁ - L₁₈₇₂) and 1 site within the interglobular domain (E₃₇₃ - A₃₇₄) [6]. Cleavage at the latter site had been documented by analysis of cartilage proteoglycan breakdown products in rheumatoid and osteoarthritis [7].

To measure aggrecanase activity, an artificial recombinant protein composed of aggrecan interglobular domain with flanking FLAG-sequence and human immunoglobulin G1 constant region was first used by Hughes et al. [8].

4. References

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