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AGGRECAN
RECOMBINANT INTERGLOBULAR DOMAIN T_{331} – G_{458} (Aggrecan-IGD1)

CATALOG NUMBER: CC1890 **QUANTITY:** 50 µg

LOT NUMBER: **CONCENTRATION:** XX mg/mL

BACKGROUND:

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 glucosaminoglycan chains are attached to the core protein and the total molecular mass can reach 2.2 – 3.0 x 10^6 Daltons [4].
Within the aggrecan molecule 3 global 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 G1domain 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 structurem, which endowes 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 aging [5]. Isolated aggrecanases cleave aggrecan at 4 sites within the chrondroitin sulfate-rich region (sites E_{1667} –G_{1668}, E_{1480}-G_{1481}, E_{1771} – A_{1772}, E_{1871} – L_{1872}) and 1 site within the interglobular domain (E_{373} – A_{374}) [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].

DESCRIPTION:

Molecular form: The polypeptide connecting human aggrecan globular domains 1 and 2 (T_{331} – G_{458}) is expressed in *E. coli* with a C-terminal His-tag. The recombinant protein contains cleavage sites for aggrecanases (E_{373} – A_{374}) and matrix metalloproteinases (N_{341} – F_{342}). It comprises the following amino acids:
T A E D F V D I P E N F F G V G G E E D I T V Q T V **T** W P D M E L P L P R N I T E G E
A R G S V I L T V K P I F E V S P S P L E P E E P F T F A P E I G A T A F A E V E N E T
G E A T R P W G F P T P G L G P A T A F T S E D L V V Q V T A V P G Q P H L P G G
(His-tag)

Main cleavage sites are indicated by arrows. The calculated M_r of the His-tagged protein is 15 493 Da.

Purity: The recombinant aggrecan interglobular domain appears as a major band at about 21 kDa in SDS-PAGE. It represents more than 90% of total protein in the preparation.

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 interlobular domain in SDS-PAGE is reduced from 21 kDa to about 13 kDa. 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.

PRESENTATION: 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 $CaCl_2$.

STORAGE/HANDLING: MT5-MMP is stable until the expiry date given on the label of 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.

REFERENCES:

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Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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