Undenatured Type II Collagen Detection

Undenatured type II collagen (UD-CII), in its natural form, has shown to improve joint health in conditions like osteoarthritis (OA) and rheumatoid arthritis (RA). Studies using a collagen-induced arthritis (CIA) model have demonstrated that consuming small amounts of UD-CII can significantly reduce inflammation by lowering levels of inflammatory cytokines in the body. UD-CII has been studied in healthy volunteers through clinical trials and has shown promising results in supporting joint health. In addition to human health, UD-CII has been used to support joint health in animals such as dogs, cats, and racehorses.

UD-CII is commonly sourced from chicken cartilage. Recently, fish cartilage, notably salmon cartilage, has also been used to prepare UD-CII. To ensure the quality and authenticity of supplements and bulk materials containing type II collagen, it is important to validate the presence of UD-CII. Chondrex, Inc.'s Type II Collagen Detection Kit (Cat # 6018) has been used to confirm the presence of native type II collagen in various sources.

1. Assay Validation

Table 1 - Reproducibility Data for the Type II Collagen Detection ELISA Kit

Test	5 ng/ml	25 ng/ml	100 ng/ml
Intra-Assay CV (%)	4.4	2.2	2.1
Inter-Assay CV (%)	9.3	6.8	6.3
Spike Test* (%)	86%	97%	97%

2. Specificity to UD-CII from Different Species

Table 2 - Reactivity of UD-CII from various species assayed by the Type II Collagen Detection ELISA Kit.

Species	Chick	Human	Mouse	Rat	Bovine	Porcine	Salmon
Cross-reactivity	100%	91%	98%	103%	247%	121%	66%

3. Specificity to UD-CII

The samples in Table 3 were prepared by mixing UD-CII and heat denatured type II collagen. The concentration of type II collagen determined by the Type II Collagen Detection Kit was almost identical to expected UD-CII concentration by the ratio of denatured and native collagen prepared.

Table 3 - Collagen Detection Rate of Samples Which Received Pepsin Treatments

Sample #	UD-CII %	Results (ng/ml)	Detection Rate
1	0	1.4	4.6%
2	25	7.1	23.4%
3	50	15.6	51.5%
4	75	21.8	71.6%
5	100	30.4	100.0%
6	100 (no pepsin)	27.7	N/A

Because pepsin only digests denatured collagen, Figure 1 presents a comparison of SDS-PAGE patterns in the pre- and post-pepsin treated samples mentioned in Table 3. Before pepsin digestion, all samples showed almost identical bands (alpha chain). After pepsin digestion, 100% denatured collagen showed almost no band and 25% denatured collagen showed a reduced band. However, there were no visible differences among 50%, 75%, and 100% native collagen, and they showed the same band as native collagen without pepsin digestion.

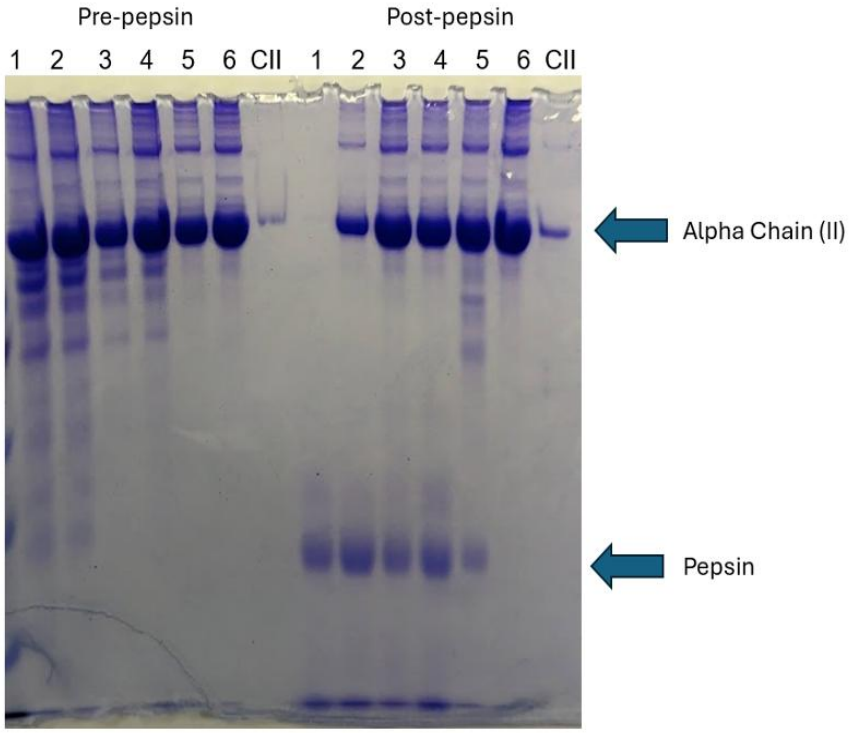

Figure 1 - A 6% SDS-PAGE analysis under non-reducing conditions for collagen solutions of constant collagen concentration, but different UD-CII content as shown in Table 3. 1-6: sample numbers and CII: type II collagen

4. Conclusion

Chondrex, Inc.'s Type II Collagen Detection Kit (Cat # 6018) shows high reproductivity in its assays, cross-reacts to type II collagen from many species, and reacts only to native (undenatured) type II collagen: UD-CII.

NOTE: Proteoglycans in Cartilage

Cartilage contains biological materials, including type II collagen and glycosaminoglycans (GAGs). One important component of GAGs, chondroitin sulfate (CS), is produced naturally in the body and can also be supplemented through diet. Supplementing CS may be especially helpful when there is an imbalance between the breakdown (catabolic) and building (anabolic) processes in cartilage.

Numerous *in vitro* and *in vivo* studies suggest that taking CS may help stabilize or improve joint conditions. Fish-derived CS particularly shows promise for osteoarthritis (OA) treatment due to its anti-inflammatory properties and ability to support cartilage regeneration. Chondrex, Inc. offers assay kits for sulfated GAGs (Cat # 6022), Hyaluronans (Cat # 6048 and 6049), and Aggrecans G1 Domain (Cat # 6058).