Analysis of Collagen, Proteoglycans, and DNA

Cartilage tissue engineering generally requires the analysis of collagen, proteoglycans such as hyaluronan or glycosaminoglycans (GAGs), aggrecans, and DNA in conjunction with cellular or extracellular matrix components (ECM) (1,2). Consequently, depending on the analytes, appropriate sample preparation protocols must be utilized. Table 1 shows three sample preparation protocols and assay kits as guidelines for ECM analysis depending on the analyte. Please contact Chondrex, Inc. at support@chondrex.com for more information.

A. Guanidine hydrochloride (GuCl) extracts GAGs, but fails to completely extract collagen and DNA, resulting in underestimated values (1,3).
B. Papain digestion solubilizes GAGs (4) and DNA (5) but degrades collagen.
C. Chondrex, Inc.'s collagen solubilization protocol can be used on samples for the GAGs assay (6), but not for the DNA assay.

To ensure accuracy, assay results can be normalized by the sample's weight, cell count, or DNA levels, according to the preparation method used (7,8).

Table 1. Sample Solubilization

Protocol		Sulfated GAGs	Aggrecans	DNA	Total Collagen	Individual Native Collagen Types
A	Guanidine Hydrochloride (GuCl) Extraction*	Yes	Yes	Yes*	No	No
B	Papain Digestion	Yes	No	Yes	Yes	No
C	GuCl Extraction*, Pepsin Digestion, and Elastase Digestion	Yes	No	No	Yes	Yes
Recommended Assays (Catalog #)		6022, 6048, 6049	6058	6023	6017	6012-16, 6018, 6019, 6021

*GuCl extraction works for samples consisting of chitosan or agarose, but not for tissues such as cartilage due to poor extraction efficiency (60%) (1).

References

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4. R. W. Farndale, D. J. Buttle, A. J. Barrett. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochem Biophys Acta* **883**, 173-177 (1986).
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Proteoglycan Assays

Chondrex, Inc. provides a sulfated GAGs Assay Kit (Cat # 6022) employing the cationic dye 1,9-dimethylmethylene blue (DMMB), which selectively binds to highly charged sulfated GAGs, excluding hyaluronan. This kit features an improved DMMB solution that reduces interference from negatively charged contaminants, such as DNA and RNA. In addition, Chondrex, Inc. provides HA assay kits that employ recombinant HA-binding proteins (HABPs) in both a competition ELISA system (Cat # 6048) and a sandwich ELISA system (Cat # 6049) for measuring HA levels in various samples, including cell culture media and serum. Furthermore, the same extraction protocol can be applied to both sulfated GAGs and HA, allowing for a single extraction followed by two analyses. Finally, Chondrex, Inc. provides a Mouse Aggrecan Detection ELISA kit that utilizes two anti-G1 domain monoclonal antibodies for measuring aggrecan levels in samples. These kits, when used together, enable comprehensive analysis of proteoglycans in biological samples.

Proteoglycan Assay Kits

Product	Sulfated GAGs	Aggrecans	Non-Sulfated HA	
Catalog Number	6022	6058	6048	6049
Detection Method	Cationic dye (DMMB)	Sandwich ELISA	Competitive ELISA	Sandwich ELISA
Analyte	Sulfated GAGs (excludes HA)	Mouse Aggrecan G1 Domain	HA	HA
Standard	Chondroitin Sulfate	Aggrecan G1 Domain	HA	HA
Sample Types	Tissue, Cultured Cells, Culture Media	Serum, Tissue, Cultured cells, Culture Media	Serum, Tissue, Cultured cells, Culture Media	Serum, Tissue, Cultured cells, Culture Media
Detection Range	3.1 - 50 µg/ml	1.6 - 100 ng/ml	16 - 1000 ng/ml	16 - 1000 ng/ml
Sample Volume (duplicate)	100 µl	100 µl	80 µl	200 µl
Validation Data	Intra-Assay: 1.7-7.6% Inter-Assay: 5.2-8% Spiking Test: 106-117%	Intra-Assay: 0.7-8.3% Inter-Assay: 5.5-8.8% Spiking Test: 92-100%	Intra-Assay: 2.8-8.6% Inter-Assay: 1.2-8.8% Spiking Test: 104-107%	Intra-Assay: 3.1-5.9% Inter-Assay: 7.8-9.8% Spiking Test: 96-109%

Sample Preparation Protocols

	Protocol	Sulfated GAGs	Aggrecans	Non-Sulfated HA
A	Guanidine Hydrochloride (GuCl) Extraction	Yes	Yes	Yes
B	Papain Digestion	Yes	No	Yes
C	GuCl Extraction, Pepsin Digestion, and Elastase Digestion	Yes	No	Yes
	Recommended Assay Kit (Catalog #)	6022	6058	6048, 6049