## 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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3. S. W. Sajdera, V. C. Hascall. Protein polysaccharide complex from bovine nasal cartilage. A comparison of low and high shear extraction procedures. *J Biol Chem* **244**, 77-87 (1969).
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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7. C. Antich, G. et al. Development of a biomimetic hydrogel based on predifferentiated mesenchymal stem-cell-derived ECM for cartilage tissue engineering. *Adv. Healthc. Mater.* **10**, e2001847 (2021).
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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%<br>Inter-Assay: 5.2-8%<br>Spiking Test: 106-117% | Intra-Assay: 0.7-8.3%<br>Inter-Assay: 5.5-8.8%<br>Spiking Test: 92-100% | Intra-Assay: 2.8-8.6%<br>Inter-Assay: 1.2-8.8%<br>Spiking Test: 104-107% | Intra-Assay: 3.1-5.9%<br>Inter-Assay: 7.8-9.8%<br>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 |