



BSI Standards Publication

**Tissue-engineered medical products —
Quantification of sulfated glycosaminoglycans
(sGAG) for evaluation of chondrogenesis**

National foreword

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Tissue-engineered medical products — Quantification of sulfated glycosaminoglycans (sGAG) for evaluation of chondrogenesis

*Produits médicaux issus de l'ingénierie tissulaire — Quantification
des glycosaminoglycannes sulfatés (sGAG) pour l'évaluation de la
chondrogenèse*



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 150, *Implants for surgery*, Subcommittee SC 7, *Tissue-engineered medical products*.

Tissue-engineered medical products — Quantification of sulfated glycosaminoglycans (sGAG) for evaluation of chondrogenesis

1 Scope

This document specifies test methods for the quantification of sulfated glycosaminoglycans (sGAG), one of the major extracellular matrix components in articular, meniscal, and elastic cartilages, and tissue-engineered cartilage constructs.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purpose of this document, the following definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

tissue-engineered cartilage

cultured construct obtained by combining living cells such as chondrocytes, or stem cells with or without scaffolds, or other types of biomaterials

3.2

sulfated glycosaminoglycans

long unbranched chains of repeating disacchararide units, which are sulfated at variable positions

EXAMPLE Chondroitin sulfate, keratan sulfate, dermatan sulfate.

3.3

test sample

piece of *tissue-engineered cartilage* (3.1) construct

3.4

parallel test sample

one of three samples divided from a *test sample* (3.3)

Note 1 to entry: The protocol provides a sGAG value from each corresponding parallel test sample.

3.5

cysteine

non-essential and sulfur containing amino acid, used for preparing digestion solutions

3.6

papain

cysteine protease enzyme, used for digesting extracellular matrices of diverse tissues

[SOURCE: Enzyme Commission (EC) Number 3.4.22.2]

3.7

1,9-dimethylmethylene blue

dye that specifically binds to a range of sulfated glycosaminoglycans ([3.2](#)), whose absorbance after binding correlates linearly with the amount of bound sGAG

3.8

chondroitin 6-sulfate

one of the sulfated glycosaminoglycans (sGAG), an important structural component of cartilage and connective tissues

4 General and principle

The quantification of sulfated glycosaminoglycans (sGAG) is ranked as one of the first steps in evaluating chondrogenesis of tissue-engineered constructs, since they are one of the primary extracellular matrix constituents of native cartilage.

sGAG can be quantified by digesting test samples with enzymes to release sGAG into solution, incubating with a sGAG-binding dye, measuring the absorbance, and normalizing to the weight (wet or dry) or DNA content of the test sample.

This document applies to tissue-engineered cartilage constructs even if the amount of sGAG content might be low and offers a dynamic range of 3 µg/ml to 100 µg/ml using a standard curve.

Binding of 1,9-dimethylmethylene blue (DMMB) dye is widely-accepted for quantifying sGAG([21](#))[[22](#)][[29](#)], and therefore was selected for this document.

The procedure to quantify sGAG in constructs comprises the following steps:

- 1) Pre-treatment;
- 2) Extraction of sGAG;
- 3) Measurement of sGAG content by the DMMB assay;
- 4) Normalization of sGAG content.

In order to realize the accurate quantification, careful attention should be paid to critical steps, such as measuring of weights and volumes, pipette manipulations, and drawing of standard curves. During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled water or water of equivalent purity (See [Annex B](#)). An example of a test report format for sGAG measurement is shown in [Annex E](#).

5 Apparatus

5.1 Clean bench.

5.2 Sterilizer, e.g. autoclave.

5.3 Phase contrast microscope.

5.4 Cell culture incubator, capable of maintaining a humidified atmosphere containing 5 % CO₂ at 37 °C.

5.5 Cell counting apparatus, e.g. hemacytometer.

NOTE If applicable, refer to ISO 20391-1.

5.6 Centrifuge.