

Background Information

What is Collagen?

Collagen is a major constituent of the extracellular matrix and the predominate protein found in animals, making up approximately 30% of all protein mass. Collagens are glycoproteins characterised by possessing at least one domain of triple helix structure.

A collagen triple helix domain is formed by 3 polypeptide α -chains which contain repeating Gly-X- Y residues, with Gly always being Glycine, and X typically representing proline and Y representing hydroxyproline. Each polypeptide chain forms a left-handed polyproline II helix, which is in turn twisted into a right-handed triple helix. The triple helix is stabilised by bonds between adjacent proline residues and between each strand.

Collagen types

At least 28 different types of collagens have been identified with Type I collagen being the most prevalent form, particularly in ligaments, tendons, skin, and bone tissue. The biophysical properties of mature, insoluble collagen allow it to withstand high tensile forces as well as being resistant to stretch. These are essential properties that enable the locomotion of organisms. Collagen also exhibits biochemical properties, being involved in cell growth, proliferation, and differentiation (*this 'biochemical' form of collagen is typically soluble in acid or acid-pepsin digestion and can be detected using the Sircol Soluble collagen assay*)

Use of Collagen as a material

Due to its various properties, extracted and purified collagen is used in many different industries. It is used in medicine for wound healing and has a continually expanding role in tissue engineering and cell culture for biomedical purposes. There is growing use in the cosmetic industry where it is used for skin rejuvenation and feedstock for chemical formulations and the food industry both as a functional food supplement and as a food additive.

Mode of Action

How does the Sircol assay detect collagen?

Sircol dye reagent contains Sirius Red - a linear anionic dye with sulphonic acid side chain groups. Under assay conditions the Sircol dye binds the basic groups of soluble collagen molecules. Maximal binding occurs in collagens possessing intact triple helix organisation as the highly ordered Gly-X-Y_n helical structure of tropocollagen further contributes to dye binding. This results in a high degree of dye-collagen specificity. Affinity is progressively reduced during heat denaturation 4°C due to the unwinding of the triple helix and formation of random chains.

Overview of the Sircol soluble collagen assay process:

Step 1. Addition of Sircol Dye Reagent to samples containing soluble collagen results in the formation of a collagen-dye complex. This complex then precipitates during the dye

incubation period and is subsequently isolated by centrifugation, followed by washing to remove unbound dye.

Step 2. Collagen-bound dye is then eluted and measured spectrophotometrically.

Step 3. The collagen content of unknown samples is quantified by comparison against a calibration curve prepared using a collagen standard supplied with the kit

Assay Specifications

Assay Specifications

Assay range

0 - 150µg/ml or 0 - 500µg/ml (depending on setup)

Limit of Detection

10µg/ml

Detection Method

Colorimetric (556nm)

Measurements per kit

110 in total (allows a maximum of 48 samples to be run in duplicate alongside a standard curve).

Suitable Samples

Soluble* collagens derived from mammals**:

In-vivo: tissues, cartilages and fluids.

In-vitro: 2D/3D culture extracellular matrices / Conditioned media.

**Collagens must be in soluble form, depending on sample this will require prior salt/acid/acid-pepsin extraction.*

***non-mammalian collagens may result in a reduced limit of detection. We recommend use of a collagen standard matched to the species under assay.*

Precautions

This kit is designed for research use only. Not for use in diagnostic procedures.

Kit requires access to a centrifuge, as well as a spectrophotometer/colorimeter capable of absorbance detection at 556nm.

Specific sample preparation protocols may require customers to provide further reagents, consult assay manual for further information.

Kit Content

The Sircol kit contains:

1. Sircol Dye Reagent (1x110ml)
2. Collagen Reference Standard (1x5ml, 0.5mg/ml soluble Bovine collagen)
3. Acid-Salt Wash Reagent (1x20ml)
4. Collagen Isolation and concentration Reagent (1x20ml)
5. Alkali Reagent (1x110ml)
6. Acid-Neutralising Reagent (1x20ml)
7. 1.5ml micro-centrifuge tubes for dye-labelling reaction.
8. Assay kit manual