

Collagen analysis:

Which collagen assay can be used for what application?

Collagens are the most abundant proteins in the vertebrate body, constituting about 30% of the total body protein. They play an important role in tissue structure and have many other functions such as in cell growth, differentiation, tissue repair and many pathological conditions.

Collagens are a family of extracellular matrix proteins; in vertebrates at least 27 collagen types with 42 distinct polypeptide chains are identified. Type I, II, III, V, XI, XXIV, XXVII collagen are fibril forming collagens, containing triple-helical structures which are able to bundle into fibrils. Characteristic for collagen is the presence of hydroxyproline residues needed for stabilization of the collagen triple helix.

Some collagens have a restricted tissue distribution, e.g. collagen types II, IX and XI are found almost exclusively in cartilage and the presence of collagen type IV is limited to basement membranes. Collagen types I, II and III are the ones that are most abundantly present in tissues.

Although in the human body more than 3 kilograms of collagen are present, collagen is a molecule which is difficult to purify and to analyze. This is partly due to the extensive network that is formed by collagen molecules via different types of crosslinking which makes the collagen molecules insoluble and difficult to extract.

Currently for the analysis of collagen various types of assays exist:

- ELISA for specific types of collagen
- ELISA for specific pro-domains of collagen
- Western blotting using specific collagen antibodies
- Sirius Red based assays for soluble collagen
- Tissue hydrolysis followed by analysis of Hydroxyproline residues (either by a colorimetric kit or by HPLC)

QuickZyme Biosciences has developed a set of assays for the analysis of collagen from any species, each with a different application area (see below)

Collagen assays available at QuickZyme Biosciences

- *Quickzyme Soluble collagen assay*
- *Quickzyme Total collagen assay*
- *Quickzyme Hydroxyproline assay*

Quickzyme Soluble collagen assay

This assay recognizes soluble or (acid/pepsin) solubilized collagen.

The assay is colorimetric, has a 96-well plate format and is based on precipitation of collagen with Sirius-Red, an anionic dye with sulphonic acid groups. This dye can bind the side-chain groups of basic amino acid residues. The dye is released from the precipitated complex at high pH followed by colorimetric detection. The assay is optimized such that other proteins (such as albumin) do not interfere. Gelatin (unfolded collagen) is not recognized by this assay.

Application: The assay is used for the measurement of (soluble) collagen in e.g. cell culture media, and (acid or acid/pepsin) solubilized collagens e.g. from cell culture extracts. The assay is less suitable for the determination of collagen in tissues, since in tissues most of the collagen is crosslinked and therefore often only a low percentage of the collagen is solubilized upon extraction.

Quickzyme Total collagen assay

This assay recognizes all types of collagen (mature, immature, procollagen, degraded collagen, crosslinked collagen, collagen from various sources).

The assay is colorimetric, has a 96-well plate format, and is based on the quantification of hydroxyproline, an amino acid exclusively occurring in collagen. Hydroxyproline is released from collagen upon acid hydrolysis of the collagen containing sample. Hydrolysis is carried out at 95 °C, and the product can directly be used for hydroxyproline analysis, without washing or drying steps. This analysis is based on Chloramine T/DMBA.

Application: The assay is used for the measurement of total collagen. This includes all procollagen, unfolded collagen, mature collagen as well as collagen degradation products of all collagen types present in the sample. Since the first step is complete hydrolysis of the sample, difficulty in extraction of collagen plays no role. The assay is applicable for all types of samples, including tissue.

Quickzyme Hydroxyproline assay

This assay is similar to the total collagen assay, with the difference that no protocols and materials are included for collagen hydrolysis.

Application: This assay has the same application area as the total collagen assay, but is intended for customers who have their own method of acid hydrolysis, or have a collection of hydrolyzed samples to be analyzed.

For more detailed information (including assay manuals) on

QuickZyme Soluble collagen assay: www.quickzyme.com/products/collagen-assay

QuickZyme Total collagen assay: www.quickzyme.com/products/total-collagen-assay

QuickZyme Hydroxyproline assay: www.quickzyme.com/products/hydroxyproline-assay