

Collagen analysis: Which 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 pro-domains of collagen
- ELISA for specific types of collagen
- Western blotting using specific collagen antibodies
- Sirius Red based staining of tissue sections
- Sirius Red based assays for soluble collagen
- Determination of Hydroxyproline residues (either by a colorimetric kit or by HPLC) after hydrolysis of the sample.

QuickZyme Biosciences has developed a set of assays for the analysis of collagen either soluble or insoluble from any species and any tissue.

Collagen assays available at QuickZyme Biosciences

- QuickZyme Soluble collagen assay
- QuickZyme Total collagen assay
- QuickZyme Sensitive Tissue Collagen Assay
- QuickZyme Hydroxyproline assay
- QuickZyme Sensitive Tissue Hydroxyproline Assay

QuickZyme Soluble collagen assay

This assay recognizes soluble or (acid/pepsin) solubilized fibrillar 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. 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 (denatured collagen) is not recognized by this assay. The assay requires a soluble or solubilized form of fibrillar collagen.

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 cellular extracts. The assay is not

recommended for tissues since most of the tissue collagen is crosslinked and insoluble.

QuickZyme Total collagen assay

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

The assay is colorimetric, has a 96-well plate format, and is based on the quantification of hydroxyproline, a modified version of the amino acid proline almost 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 hydrolysate can directly be used for hydroxyproline analysis, without washing or drying steps.

Application: The assay is used for the quantification of total collagen. This includes all procollagen, unfolded collagen, mature collagen as well as collagen degradation products and gelatin 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 from any species. This assay is influenced by matrix effects due to unidentified substances in tissue samples. The effects can in many samples be prevented by dilution of the hydrolysate. If sufficient dilution of the hydrolysate is not desirable due to low collagen concentration such as occurs e.g. in liver tissue, we recommend our Sensitive Tissue collagen assay

QuickZyme Hydroxyproline assay

This assay is similar to the total collagen assay, with the difference that protocols and tubes for collagen hydrolysis are not included.

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

QuickZyme Sensitive Tissue collagen assay

This assay is very similar to the QuickZyme Total collagen assay, but is not influenced by matrix effects making it the assay of choice for tissues such as liver tissue having low collagen concentrations and substantial matrix effects.

Application: The assay is used for the measurement of total collagen in (tissue) samples with low concentration of collagen and matrix effects that would require dilution in the QuickZyme Total collagen assay

QuickZyme Sensitive Tissue Hydroxyproline assay

This assay is similar to the QuickZyme Sensitive Tissue collagen assay, with the difference that protocols and tubes for collagen hydrolysis are not included.

Application: This assay has the same application area as the QuickZyme Sensitive Tissue 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 these assays see the support section on our website: www.quickzyme.com/support/

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