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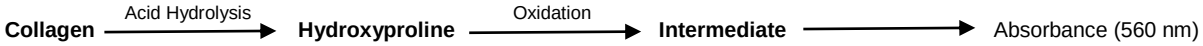
Total Collagen Assay Kit (Colorimetric)

Rev 07/19

(Catalog # K218-100; 100 assays; Store at -20°C)

I. Introduction:

Collagen is the most abundant insoluble protein found in the extracellular matrix and connective tissues. It can be found in skin, tendons, bone, cartilage, muscle, vitreous humor and ligaments, among other tissues. There are more than sixteen - well characterized types of collagens, but types I, II and III collagen comprise more than 80% content in mammals. The triple-helical structure of collagen is quite unique: it consists of a repeating pattern of a basic trimer: Glycine-Proline-Hydroxyproline. In cells, collagens are secreted as procollagens and these chains are transported into the Endoplasmic Reticulum, where, numerous post-translational modifications lead to the formation of a triple helix with disulfide bonds. Excessive production of collagen is linked to pathological conditions including liver cirrhosis, lung fibrosis, and tumor growth. BioVision's Collagen Assay Kit is a simple and sensitive assay to detect small amounts of collagens in a variety of samples. The assay is based on the acid hydrolysis of samples to form hydrolysates and Hydroxyproline. This released Hydroxyproline gets oxidized to form a reaction intermediate, which further in the reaction, forms a chromophore (Abs 560 nm). The assay is simple, sensitive and specific for collagen and can detect as low as 0.5 µg of collagen in a variety of samples such as tissue homogenates, biological fluids and purified proteins.



II. Application:

- Measurement of collagen in various sample types.

III. Sample Types:

- Mammalian tissues
- Protein/peptide hydrolysates
- Serum
- Urine

IV. Kit Contents:

Components	K218-100	Cap Code	Part Number
Oxidation Buffer	10 ml	WM	K218-100-1
Chloramine T Concentrate	0.6 ml	Red	K218-100-2
Perchloric Acid/Isopropanol Solution	5 ml	NM	K218-100-3
DMAB Concentrate (in DMSO)	5 ml	Amber	K218-100-4
Collagen I Standard (2 mg/ml)	0.1 ml	Yellow	K218-100-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- 12 M Hydrochloric Acid (Concentrated HCl)
- For hydrolysis: Polypropylene Vials (BV Cat. No. M1352) and Screw Caps (BV Cat. No. M1353)

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Chloramine T Reagent:** For each well to be analyzed, add 6 µl of Chloramine T Concentrate to 94 µl of Oxidation Buffer and mix well.
- **DMAB Concentrate (in DMSO):** For each well to be analyzed, add 50 µl of DMAB Concentrate to 50 µl of Perchloric acid/Isopropanol Solution and mix well. Keep on ice, protected from light.

  Note: The reagent concentrates are stable as supplied. Once the concentrates have been diluted to working concentration, they are only stable for 1-2 hr, so prepare fresh reagents as necessary for the number of Samples and Standards to be quantified.

VII. Collagen Assay Protocol:

1. **Sample Preparation: Tissue or protein/peptide samples:** tissue samples (*i.e.* lung) should be homogenized in ddH_2O, using 100 µl ddH_2O for every 10 mg of tissue. To a 100 µl of sample homogenate, add 100 µl concentrated HCl (~12 M, not provided) in a pressure-tight polypropylene screw-capped vial. Hydrolyze Samples at 120°C for 3 hours (**see note c**). **Urine:** hydrolyze Samples with equal volumes of 12 M concentrated HCl (*i.e.* 100 µl Urine + 100 µl HCl) in a pressure-tight polypropylene screw-capped vial. After homogenization, clarify Samples with activated charcoal by adding 4 mg of activated charcoal. Vortex and centrifuge at 10000 x *g* for 3 min to remove precipitate and activated charcoal. Repeat if needed. Transfer 2-30 µl of each hydrolyzed Sample to a 96-well plate and evaporate to dryness by heating the plate at 70°C on a hot plate/dry heat block or microplate incubator.

   Notes:

   a. For Unknown Samples, we recommend performing a pilot experiment to ensure readings are within the standard curve range and adjusting the volume of Sample hydrolysate accordingly (2-30 µl of hydrolysate may be used) or diluting hydrolysate if necessary.

   b. For Samples with an extremely low collagen concentration, we recommend running two test Sample wells in parallel and spiking one with a known amount of Collagen I Standard (4.0 µg) to ensure accurate determination.

   c. For Sample hydrolysis, polypropylene vials with tight-fitting screw-on caps (without O-rings) yield best results. We recommend Biovision's Polypropylene Vials and Caps (Cat. No. M1353 and M1352).

2. **Standard Preparation:** Transfer 25 µl of the Collagen I Standard to a pressure-tight, screw-capped polypropylene vial and add 25 µl of 12 M concentrated HCl (not provided). Securely tighten cap and hydrolyze at 120°C for 3 hr. Cool vial on ice, then spin down the vial contents (the final volume will be 50 µl, with a final concentration of 1 mg/ml hydrolyzed collagen). Add 0, 2, 4, 6, 8, and 10 µl of the 1 mg/ml hydrolyzed Collagen I Standard solution into a series of wells in a 96-well plate, generating the equivalent of 0, 2, 4, 6, 8 and 10 µg of collagen/well. Evaporate the Standard Curve wells to dryness by heating the plate at 70°C on a hot plate/dry heat block or microplate incubator.

   Note: To prevent warping/etching of the microplate plastic, do not expose the microplate to extreme temperatures (>85°C). If a hot plate/oven is used, we recommend placing the microplate at 70°C until the contents are completely evaporated

3. **Reaction:** Add 100 µl of the Chloramine T reagent to each Sample and Standard and incubate at room temperature for 10 min. Add 100 µl of the DMAB reagent to each well and incubate for 90 min at 60°C.

4. **Measurement:** Remove the plate from the heat source and measure the absorbance of all Sample and Standard Curve wells at 560 nm (OD_{560}) in endpoint mode. *For maximum signal intensity, measure the absorbance within 20 min of removing plate from the heat source.*

4. **Calculation:** For the Collagen Standard Curve, subtract the zero Standard (0 µg/well reagent blank) reading from all Standard readings, plot the Background-subtracted values and calculate the slope of the Standard Curve. For Test Samples, subtract the zero Standard (0 µg/well reagent blank) reading from Sample readings and apply the Background-subtracted OD_{560} values to the Standard Curve to get *B* µg of collagen in the well.

$$\textbf{Sample Hydrolysate Collagen Concentration (C)} = \frac{B}{V} \times D = \text{µg/µl}$$

Where: ***B*** is the amount of collagen, calculated from the standard curve (in µg)
V is the volume of Sample hydrolysate added to the well (in µl)
D is the *post-hydrolysis* Sample dilution factor (if applicable, *D*=1 for undiluted samples)

Note: For spiked samples, correct for any sample interference by using the following equation:

$$\textbf{Collagen amount in spiked sample well (}B\textbf{)} = \frac{\text{OD}_{\text{sample (corrected)}}}{(\text{OD}_{\text{sample+spike (corrected)}})-(\text{OD}_{\text{sample (corrected)}})} * \text{Collagen spike (µg)}$$

Hydroxyproline MW: 131.13 g/mol
Hydroxyproline Content in Type I Collagen (from rat tail): 12-14% by weight

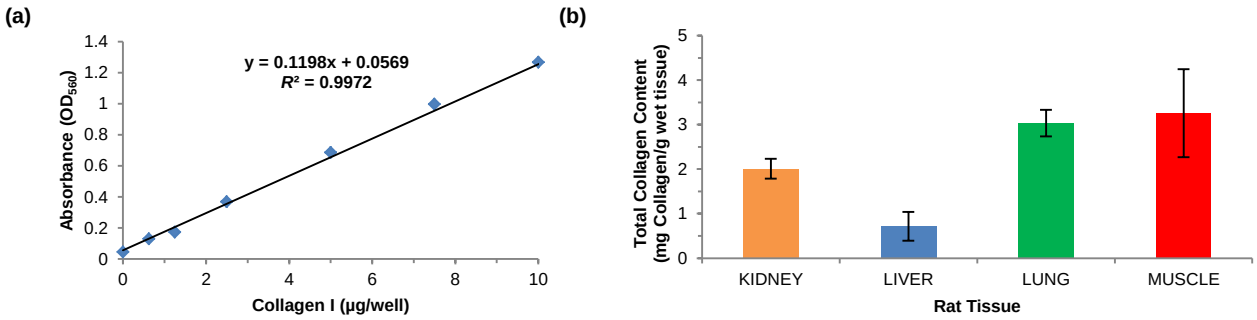


Figure: (a) Hydrolyzed Collagen I Standard Curve (0-10 µg). **(b)** Estimation of Total Collagen Concentration in rat tissues. Rat kidney, liver, lung and muscle samples were homogenized with ddH_2O and hydrolyzed with 12 M HCl for 3 hours at 120°C. Precipitates were removed by centrifugation (10000 x *g* for 3 min.). Thirty microliters of the hydrolyzed samples were assayed according to the kit protocols. Collagen Content (mg Collagen/g wet tissue): Kidney: 2.01 ± 0.22; Liver: 0.71 ± 0.32; Lung: 3.03 ± 0.3; Muscle: 3.25 ± 0.98.

VIII. Related Products:

Collagenase Activity Colorimetric Assay Kit (K792)
Hydroxyproline Colorimetric Assay Kit (K555 and K226)
Collagen-I (4796)
Collagenase Inhibitor Screening Kit (Fluorometric) (K833)
Soluble Collagen Assay Kit (Fluorometric) (K532)
Collagen-III (4797)

FOR RESEARCH USE ONLY! Not to be used on humans.