

Technical Data Sheet (TDS)

Product Name: Col Quant™ - Total Collagen Quantification Kit

Catalogue Number: BVCQ96

1. Product Introduction:

Accurate measurement of collagen is critical in fields such as fibrosis research, tissue engineering, wound healing, biomaterial development, and drug discovery. ColQuant™ Total Collagen Quantification Kit provides a robust, sensitive, and reproducible method to estimate total collagen by measuring hydroxyproline. The simple, microplate-based ColQuant™ Quantification Kit allows for high-throughput analysis and ensures accurate quantification with minimal hands-on time.

2. Assay Principle (Colorimeter, 560 nm)

Hydroxyproline is a primary, post translationally modified amino acid of collagen, making its presence a definitive marker for the protein. The sample is initially hydrolysed to release free hydroxyproline (HYP). The released hydroxyproline then undergoes oxidation in the presence of an oxidizing agent to form a stable product. This oxidation product subsequently reacts with a chromogenic reagent yielding a reddish-purple complex. The hydroxyproline concentration is then determined by measuring the optical density (OD) at 560 nm.

3. Key Features & Performance

Feature	Specification
Assay Type	Colorimetric (microplate, endpoint)
Readout	Absorbance at 560 nm
Sensitivity	As low as 0.05 µg Hyp/well (typical)
Linear Range	0.2 – 1.0 µg Hyp/well (typical)
Assay Time (post-hydrolysis)	≈ 60–90 min
Samples	Tissue lysates, cultured cells/ECM, serum, plasma, urine
Applications	Total collagen quantification; fibrosis and ECM studies; biomaterial validation; wound-healing models
Format	96-well

Technical Data Sheet (TDS)

4. Kit Components

Component	Qty	Storage
Reagent 1	10mL x 1 Vial	2-8°C, 12 months
Reagent 2	2 mL x 1 Vial	2-8°C, 12 months
Reagent 3	8 mL x 1 Vial	2-8°C, 12 months
Reagent 4	40 mL x 1 Vial	2-8°C, 12 months
Reagent 5	50 mL x 1 Vial	2-8°C, 12 months
Reagent 6	50 mL x 1 Vial	2-8°C, 12 months
Standard	5 mg x 1 Vial	2-8°C, 12 months
96 well plate – Standard Clear Bottom	1	RT
Plate Sealing Film	1	RT
Technical Data Sheet	1	-
Certificate of Analysis (CoA)	1	-

Note: Reagents must be stored strictly under the preservation conditions specified in the table above, and reagents from different kits should not be mixed. For small-volume reagents, centrifuge briefly before use to ensure sufficient recovery of the reagent.

5. Technical Specifications

Parameter	Details
Catalog No.	BVCQ96
Wavelength	560 nm
Required Equipment	Microplate reader (560 nm), dry heat block/oven (120 °C, 65 °C)
Hydrolysis	Acid (6 N HCl) hydrolysis prior to assay
Sample Types	Tissue, cell lysate/ECM, serum, plasma, urine
Shelf Life	12 months at recommended storage
Compliance	Research Use Only (RUO)

Technical Data Sheet (TDS)

6. Materials and equipment required but Not Supplied

Instruments

- Autoclave
- Vortex mixer
- Centrifuge
- Water bath
- Microplate Reader capable of reading 560 nm

Reagents

- 6N NaOH
- 6 N HCl

Reagents preparation

- Equilibrate all reagents to room temperature before use.
- **The preparation of 1 mg/mL HYP standard**
Dissolve one vial of HYP Standard with 5 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 15 days.
- **The preparation of 100 µg/mL HYP standard**
Dilute 40 µL of a 1 mg/mL HYP standard solution with 360 µL of double-distilled water and mix thoroughly. The 100 µg/mL HYP standard should be freshly prepared prior to use.
- **The preparation of Oxidant working solution**
Dilute Reagent 2 and 3, mix well to dissolve. Store at 2-8°C and protected from light.
- **The preparation of standard curve**
 - Always prepare a fresh set of standards and discard working standard dilutions after use.
 - Dilute 100 µg/mL standard solution with double distilled water to a serial concentration.
 - The recommended dilution gradient is as follows: 0, 1, 3, 4, 6, 8, 10 µg/mL.

Technical Data Sheet (TDS)

Reference is as follows:

Item	1	2	3	4	5	6	7
Concentration (µg/mL)	0	2	3	4	6	8	10
100 µg/mL standard solution (µL)	0	20	30	40	60	80	100
Double distilled water (µL)	1000	980	970	960	940	920	900

Sample Preparation

Collagen: Hydrolysis of Collagen Samples (Example)

- Weigh 3 mg of sample collagen and 3 mg of pure collagen separately using an analytical balance and transfer each into a clean 5 mL glass vial.
- Add 1 mL of 6N HCl to each vial to obtain a concentration of 3 mg/mL.
- Close the vial caps gently (do not overtighten).
- Autoclave (hydrolyze) the samples at 121°C for 60 minutes.
- Allow the samples to cool at room temperature for 1-2 hrs.

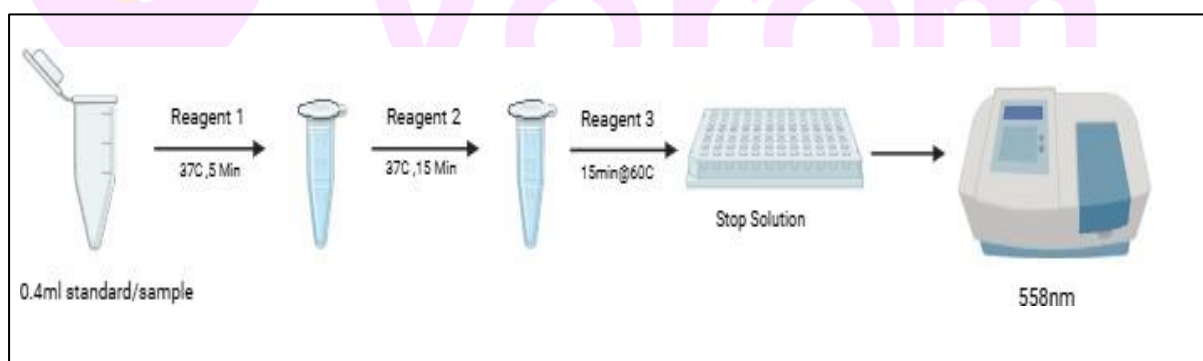
Neutralization

- Neutralize each hydrolyzed sample with 6N NaOH until the pH reaches neutral (≈ 7.0) to prevent further amino acid degradation.
- Adjust the final solution volume to 2 mL, resulting in a 1.5 mg/mL concentration.
- Store the neutralized solutions at 4°C until further use.

Technical Data Sheet (TDS)

7. Operating Steps

- **Standard tube:** Add 400 μ L of each standard solution prepared earlier with different concentrations and add to a separate 2 mL tube.
- **Sample tube:** Take 400 μ L of sample to the 2 mL EP tube.
- Add 100 μ L of Reagent 1 solution to each tube.
- Add 100 μ L of oxidant working solution to each tube.
- Mix fully and stand at room temperature for 15 minutes.
- Add 400 μ L of chromogenic working solution to each tube.
- Mix fully and incubate the tubes at 60°C for 15 minutes.
- Cool the tubes to room temperature with running water, then transfer the contents to a 96 well plate.
- Measure the OD value of each well at 560 nm with a spectrophotometer.



8. Calculations

Hydroxyproline and Total Collagen Calculations:

1. Calculate Hydroxyproline in Samples

Sample Hyp (μ g/well):

$$\text{Sample Hyp} = (\text{Interpolated value from the standard curve}) \times f$$

Technical Data Sheet (TDS)

Where:

- Interpolated value = Hydroxyproline amount corresponding to the sample OD based on the standard curve.
- f = Dilution factor applied to the sample before testing.

2. Estimate Total Collagen

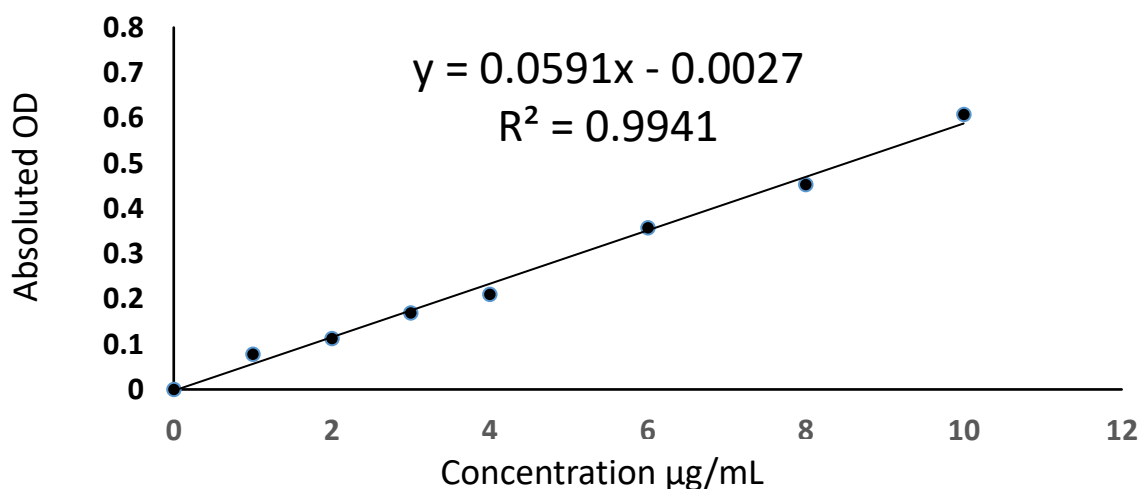
Collagen ($\mu\text{g}/\text{well}$):

Collagen = sample Hyp $\times$ Conversion factor

Where:

- Conversion factor = 7.46 (typical value), with an acceptable range of 7.14-7.69 depending on tissue factor.

Standard Curve:



9. Storage & Safety

Store the kit at $-20\text{ }^{\circ}\text{C}$ (Developer at $4\text{ }^{\circ}\text{C}$). Protect DMAB and Chloramine-T from light. Handle all reagents with standard PPE; follow local regulations for disposal. RUO—Not for diagnostic use.

Technical Data Sheet (TDS)

10. Contact Information

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