EnzyFluo™ Collagen Assay Kit (ECOL-100)

Quantitative Fluorimetric Collagen Determination

DESCRIPTION

COLLAGEN is the key structural protein of connective tissue and the most abundant protein in mammals. It occurs in many different types and forms with Types I -V being the most common. Aside from the crucial role it plays in the body, it has numerous medical applications such as its use in reconstructive surgery including bone and skin grafts. It is also commonly used in cosmetics due to its anti-aging and skin healing properties. Assay methods available for quantifying collagen currently range from needing extensive hydrolysis procedures with acids and bases to using expensive antibodies and complicated protocols.

BioAssay Systems' collagen assay kit delivers a very simple, safe, and sensitive method to quantify collagen in samples. In the first step of this procedure, collagen in the sample is enzymatically digested into peptides. Subsequently, the N-terminal glycine containing peptides react with the dye reagent to form a fluorescent complex. The fluorescence intensity of this product, measured at $\lambda_{ex/em}$ = 375/465 nm, is directly proportional to collagen concentration in the sample.

KEY FEATURES

Safe. Non-radioactive assay.

Sensitive and accurate. Use of 20 µL sample. Linear detection range 2 µg/mL to 50 µg/mL collagen in 96-well plate assay.

Convenient and high-throughput. "Add-mix-read" type assay. No wash and reagent transfer steps are involved. Can be readily automated to process thousands of samples per day.

APPLICATIONS

Direct Assays: Collagen in biological samples. Collagen in cosmetic products.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Dye Reagent:	5 mL	**Collagen Standard:**	40 µL (3 mg/mL)
Buffer:	5 mL	**Developer:**	1 mL
Digest Enzyme:	70 µL		

Storage conditions. The kit is shipped on ice. Store kit components (except Collagen Standard) at -20°C upon receiving. Collagen Standard is stored at 4°C. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation: This assay is optimized for detection of very low levels of collagen. Due to the assay's sensitivity and the abundance of collagen, large dilution factors are often needed to place samples within the linear detection range of this kit. A serial dilution to determine the optimal dilution factor for a type of sample is highly recommended.

Biological fluid samples (e.g. serum, cell lysate, tissue lysate) first centrifuge to remove any particulates. A serial dilution may be needed to determine the optimal dilution factor for each type of sample in this assay.

Procedure using 96-well plate

1. *Standards*. Prepare 50 µg/mL Collagen Standard by mixing 5 µL 3 mg/mL Collagen Standard and 295 µL dH_2O. Next prepare standards in 1.5-mL centrifuge tubes as described in the Table.

No	50 µg/mL Standard + H_2O	Collagen (µg/mL)
1	100 µL + 0 µL	50
2	60 µL + 40 µL	30
3	30 µL + 70 µL	15
4	0 µL + 100 µL	0

2. Transfer 20 µL Standards into separate wells of a black, flat-bottom 96-well plate. Transfer 20 µL of each sample *in duplicate* into separate wells (one well as "Sample" and one well as "Sample Blank").
3. Prepare sufficient Digest Mix for the Standards and the "Sample" wells by mixing, for each well: 35 µL Buffer and 0.5 µL Digest Enzyme.

   Add 30 µL Digest Mix to the Standards and the "Sample" wells.

   Add 30 µL Buffer to the "Sample Blank" wells.

   Tap plate to mix briefly and thoroughly. Cover plate and incubate 60 minutes at 37°C.
4. Add 40 µL Dye Reagent to all wells. Incubate 10 minutes at 37°C.
5. Add 8 µL Developer to all wells. Incubate 10 minutes at 37°C.
6. Read Fluorescence at $\lambda_{ex/em}$ = 375/465 nm.

CALCULATION

Subtract the blank value (#4) from the standard values and plot the ΔF against standard concentrations. Determine the slope and calculate the collagen concentration of Sample,

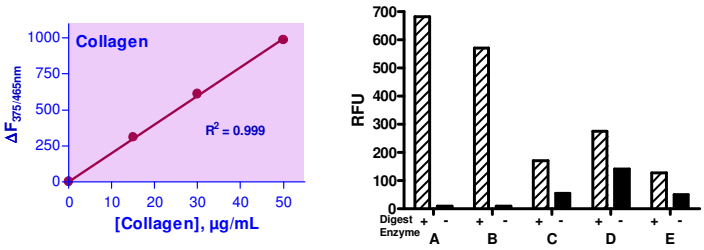
$$[\text{Collagen}] = \frac{F_S - F_B}{\text{Slope } (\mu g/mL^{-1})} \times n \quad (\mu g/mL)$$

where F_S and F_B are fluorescence readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.
Note: if the sample F value is higher than F for the 50 µg/mL *Collagen Standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.*

Conversions: 50 µg/mL equals 5mg/dL, or 50 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, black flat-bottom 96-well plates, and plate reader.



Collagen Assay in 96-Well Plate

Left: Standard curve in 96-well plate assay
Right: Collagen Samples with (+) and without (-) Digest Enzyme. A) Collagen Standard 30 µg/mL. B) Essential Wholesale Marine Collagen, 250-fold diluted. C) Rat Serum, 20-fold diluted (2.7 mg/mL protein). D) Bovine Serum, 10-fold diluted (7.0 mg/mL protein). E) Human Serum, 20-fold diluted (3.14 mg/mL protein).

LITERATURE

1. Inoue, Y., et al (2012). Accelerating effect of soy peptides containing collagen peptides on type I and III collagen levels in rat skin. Biosci Biotechnol Biochem 76(8): 1549-51.
2. Kinoshita, J., et al (2010). Type IV collagen levels are elevated in the serum of patients with peritoneal dissemination of gastric cancer. Oncol Lett 1(6): 989-994.
3. Seo, H.Y., et al. (2014). Phospholipase D1 decreases type I collagen levels in hepatic stellate cells via induction of autophagy. Biocehm Biophys Res Commun 449(1): 38-43.
4. Di Lulio, G.A., et al (2002). Mapping the ligand-binding sites and disease associated mutations on the most abdundant protein in the human, type I collagen. J Biol Chem 277 (6): 4223-4231.