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Product Information

Collagen Assay Kit

Catalog Number **MAK322**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product 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, collagen 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 those needing extensive hydrolysis procedures with acids and bases to others using expensive antibodies and complicated protocols.

The Collagen Assay Kit delivers a very simple, safe (non-radioactive), 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 λ_{ex} = 375/λ_{em} = 465 nm, is directly proportional to collagen concentration in the sample.

This kit can be used for collagen determination in biological and cosmetic products.

Components

The kit is sufficient for 100 fluorometric assays in 96 well plates.

Dye Reagent Catalog Number MAK322A	5 mL
Buffer Catalog Number MAK322B	5 mL
Digest Enzyme Catalog Number MAK322C	70 µL
Collagen Standard Catalog Number MAK322D	40 µL
Developer Catalog Number MAK322E	1 mL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Black, flat bottom 96 well plates
- Centrifuge tubes
- Fluorescence plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reagent Preparation

Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior to assay.

Storage/Stability
The kit is shipped on dry ice. Store kit components (except Collagen Standard) at –20 °C upon receiving. Collagen Standard is stored at 4 °C.

Procedure
Use ultrapure water for the preparation of all standards and samples.

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

Table 1.
Preparation of Collagen Standards

Tube	50 µg/mL standard	Ultrapure water	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

Transfer 20 µL of each standard into separate wells of a black, flat bottom 96 well plate.

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. For biological fluid samples (e.g., serum, cell lysate, or tissue lysate), first centrifuge to remove any particulates. After centrifugation, perform a serial dilution to determine the optimal dilution factor for each type of sample in this assay.

Transfer 20 µL of each sample in duplicate into separate wells (one well as "Sample" and one well as "Sample Blank").

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 2. 30 µL of the Master Reaction Mix is required for each Standard and "Sample" well.

Table 2.
Master Reaction Mix

Reagent	Stds and Samples Volume
Buffer	35 µL
Digest Enzyme	0.5 µL

2. Add 30 µL of the Master Reaction Mix to the Standards and the "Sample" wells.
3. Add 30 µL of Buffer to the "Sample Blank" wells.
4. Tap plate to mix briefly and thoroughly. Cover plate and incubate 60 minutes at 37 °C.
5. Add 40 µL of Dye Reagent to all wells. Incubate 10 minutes at 37 °C.
6. Add 8 µL of Developer to all wells. Incubate 10 minutes at 37 °C.
7. Read Fluorescence at λ_{ex} = 375/λ_{em} = 465 nm.

Results
Subtract the blank value (Standard Tube 4) from the values of the other standards and plot the ΔF against standard concentrations. Determine the slope and calculate the collagen concentration of Sample(s).

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

F_S = fluorescence reading of the Sample
F_{SB} = fluorescence reading of the Sample Blank
n = the sample dilution factor

Note: If the sample fluorescence value is higher than fluorescence for the 50 µg/mL Collagen Standard, dilute sample in ultrapure water and repeat the assay.

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

Figure 1.
Example of standard curve in 96 well plate assay

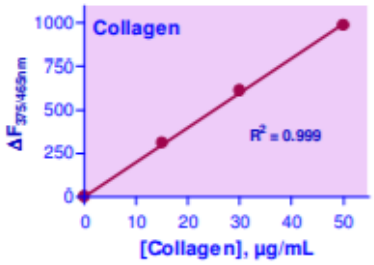
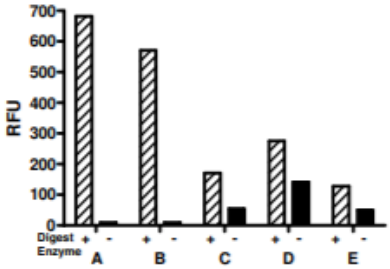


Figure 2.
Sample and Sample Blank Fluorescence



Collagen Samples with (+) and Sample Blanks 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)

References
1. Inoue, Y. et al., 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 (2012).
2. Kinoshita, J. et al., Type IV collagen levels are elevated in the serum of patients with peritoneal dissemination of gastric cancer. Oncol. Lett., **1(6)**, 989-994 (2010).
3. Seo, H.Y. et al., Phospholipase D1 decreases type I collagen levels in hepatic stellate cells via induction of autophagy. Biochem. Biophys. Res. Commun., **449(1)**, 38-43 (2014).
4. Di Lulio, G.A. et al., 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 (2002).

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