Product Information Sheet

Soluble Collagen Quantification Assay Kit

Fluorometric

CS0006

Product Description

Collagen is one of the most abundant proteins in connective tissues and internal organs of mammals. Collagen provides the tensile strength of the extracellular matrix (ECM) and is classified into several structurally and genetically distinct types. Although different types of collagen exist, they are all composed of molecules with three polypeptide chains that are arranged in a triple helical conformation. Slight differences in the primary structure (amino acid sequence) establish differences between the types.[1-4]

The Soluble Collagen Quantification Assay Kit provides a simple and sensitive procedure for measuring soluble collagen in various sample types. The kit does not require the use of perchloric acid. The amount of soluble collagen is determined based on an enzymatic reaction, where collagen is specifically digested into peptides. Subsequently, the collagen peptides are labeled with a fluorescent probe. The fluorescence intensity, measured at λ_{ex} = 375 nm / λ_{em} = 465 nm, is proportional to the amount of soluble collagen in the sample.

This kit can be used to quantify soluble collagen[5] extracted from tissues (such as muscle or heart), tissue culture cell lysate and medium, serum samples, collagen in food, and purified collagens of various sources. This kit can detect purified collagen types I, II, III, IV and V.

Precautions and Disclaimer

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.

Storage/Stability

The kit is shipped on dry ice. Upon receipt, store all components at –20 °C, protected from light. Upon thawing, the Assay Buffer and 10× Collagen Standard should be stored at 2-8 °C. The unopened kit is stable for 2 years as supplied.

Components

This kit contains sufficient reagents for 200 fluorometric tests in 96-well plates.

Component	Component Number	Amount	Cap Color/ Component Information
Assay Buffer	CS0006A	50 mL	White cap/ bottle
10× Collagen Standard	CS0006B	300 µL	Yellow cap/ vial
5× Digest Enzyme	CS0006C	1 mL	Red cap/ vial
30× Probe	CS0006D	600 µL	Brown vial
10× Development Solution	CS0006E	600 µL	Brown vial

Component Information

Assay Buffer (Component CS0006A): Ready-to-use. Upon thawing, store at 2-8 °C.

10× Collagen Standard (Component CS0006B): Contains a 2 mg/mL Collagen Type I solution. Upon thawing, store at 2-8 °C.

5× Digest Enzyme (Component CS0006C): Store at –20 °C. To avoid multiple freeze/thaw cycles, it is recommended to prepare aliquots upon thawing, and store the aliquots at –20 °C. Keep on ice while in use.

30× Probe (Component CS0006D): Prior to use, vortex thoroughly. To avoid multiple freeze/thaw cycles, it is recommended to prepare aliquots upon thawing, and store the aliquots at –20 °C, protected from light.

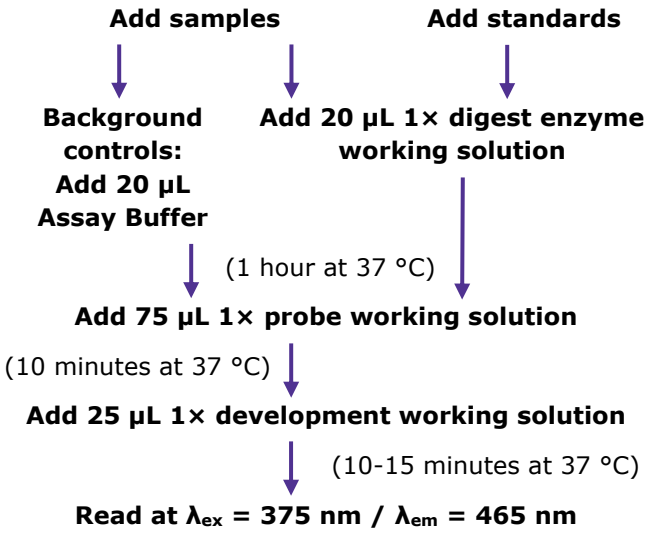
10× Development Solution (Component CS0006E): Prior to use, vortex thoroughly. To avoid multiple freeze/thaw cycles, it is recommended to prepare aliquots upon thawing, and store the aliquots at –20 °C, protected from light.

Equipment Required (Not Provided)

- 96-well black flat-bottom plates
- Fluorescence (λ_{ex} = 375 nm / λ_{em} = 465 nm) plate reader
- 0.5 M acetic acid (if required; see "Sample preparation" below)
- 0.5 M NaOH (if required; see "Sample preparation" below)

Procedure

Summary (flowchart)



General Notes

- All samples, background controls, and standards should be run in duplicate.
- A fresh set of standards should be prepared for each set of assays.
- Briefly centrifuge vials before opening.
- All reagents except the 5× Digest Enzyme should be equilibrated to room temperature before use. The 5× Digest Enzyme should be kept on ice while in use.
- **For convenience, an Excel-based calculation sheet is available on the Product Detail Page.** Use this sheet to calculate the amounts of reagents required, as well as to calculate the test results.
- All assays (samples, standards, and blank) require 80 µL of sample for each reaction (well). Therefore, bring the volume to 80 µL if required. When required, samples should be diluted in Assay Buffer. For unknown samples, it is suggested to test several sample dilutions to ensure that the readings are within the linear range of the standard curve.
- A background control should be included for each tested sample (see details below).
- The kit's optimal pH range is 7.5-8.0. If samples do not fall within this pH range, it is suggested to adjust the pH.

Sample preparation

Adherent cells:

1. Remove culture medium.
2. Trypsinize cells.
3. Collect the harvested cells by centrifugation.
4. Wash cells in PBS.
5. Pellet cells by centrifugation and aspirate PBS.
6. Resuspend cell pellet in 1 mL ice-cold 0.5 M acetic acid per ~1 × 10^7 cells.
7. Collagen can be extracted by sonicating the lysate on ice for several sonication cycles, to achieve a homogeneous preparation. To ensure the availability of soluble collagen for the assay, it is important to keep samples chilled during the sonication procedure.
8. Transfer the sample to a microfuge tube. Vortex thoroughly. Incubate at 4 °C overnight with gentle agitation.
9. Centrifuge the sample at 10,000 × g for 15 minutes at 4 °C. Transfer the supernatant to a new microfuge tube.
10. Neutralize the sample by adding an equal volume of 0.5 M NaOH to the supernatant.

Secreted collagen from cell culture:

Note: These samples can be assayed directly.

1. Collect a sample of culture medium. If the cells are in suspension, centrifuge to remove the cells, and collect the supernatant.
2. Centrifuge at 10,000 × *g* for 15 min at 4 °C to pellet any cells and/or debris.
3. Collect the supernatant, to be used in the assay.

Serum samples:

- Note: These samples can be diluted.
- Typical dilutions are in the range of 10-20 fold in Assay Buffer. However, it is suggested to test several sample dilutions to ensure that the readings are within the linear range of the standard curve.

Soft tissues:

1. Soft tissue samples should be rinsed with ice-cold ultrapure water or PBS to remove any residual blood.
2. Blot dry.
3. Dissociate the tissue with scissors to obtain small, fine pieces.
4. Add 1 mL of ice-cold 0.5 M acetic acid per ~100 mg of the dissociated tissue.
5. To extract the collagen, the dissociated tissue can be sonicated on ice for several sonication cycles, to achieve a homogeneous preparation.
6. To ensure the availability of soluble collagen for the assay, it is important to keep samples chilled during the sonication procedure.
7. The sample should be transferred to a microfuge tube, vortexed thoroughly, and incubated at 4 °C overnight with gentle agitation.
8. Centrifuge the homogenate at 10,000 × *g* for 15 minutes at 4 °C.
9. Transfer the supernatant to a new microfuge tube.
10. Neutralize the sample by adding an equal volume (such as 1 mL) of 0.5 M NaOH to the supernatant.

Collagen standard curve preparation:

Dilute the 10× Collagen Standard (yellow cap vial) 10-fold to a final concentration of 0.2 mg/mL: 20 µL of the 10× Collagen Standard with 180 µL of ultrapure water, to prepare a 1× collagen standard.

Add 0, 1, 2, 4, 6, 8, and 10 µL of the 1× collagen standard into a 96-well plate, to generate 0 (blank), 0.2, 0.4, 0.8, 1.2, 1.6, and 2 µg/well standards. Complete the volume to 80 µL with Assay Buffer (see Table 1).

Table 1. Preparation of Collagen Standards*

1× collagen standard volume	Assay Buffer volume	Final collagen amount per well
0 µL	80 µL	0 µg (blank)
1 µL	79 µL	0.2 µg
2 µL	78 µL	0.4 µg
4 µL	76 µL	0.8 µg
6 µL	74 µL	1.2 µg
8 µL	72 µL	1.6 µg
10 µL	70 µL	2 µg

* Work in duplicate

Digest Enzyme

1. Dilute the 5× Digest Enzyme (red cap vial) 5-fold in Assay Buffer to prepare a 1× digest enzyme working solution, according to Table 2. 20 µL of the 1× digest enzyme working solution is required for each reaction (well).

   Note: Include a sample background control (by replacing the digest enzyme with Assay Buffer) **for each sample**. The standard curve does not require a background control. Multiply the volumes in Table 2 according to the number of wells in the assay.

Table 2. Preparation of 1× digest enzyme working solution, per one well

	5× Digest Enzyme volume	Assay Buffer volume	1× Digest Enzyme working solution final volume
Sample and standards	4 µL	16 µL	20 µL
Sample background control (for samples only)	–	20 µL	–

2. Add 20 µL of the 1× digest enzyme working solution to each of the standard and sample wells.
3. Add 20 µL of sample background control (Assay Buffer) to each of the sample background control wells.
4. Mix well. Incubate for 60 minutes at 37 °C.

Probe

1. Immediately prior to use, dilute the 30× Probe 30-fold in Assay Buffer, to prepare a 1× probe working solution, according to Table 3. 75 µL of the 1× probe working solution is required for each reaction (well). Multiply the volumes in Table 3 according to the number of wells in the assay.

Table 3. Preparation of 1× probe working solution, per one well*

30× Probe volume	Assay Buffer volume	1× probe working solution final volume
2.5 µL	72.5 µL	75 µL

* Protect from light

2. Add 75 µL of the 1× probe working solution to each of the standard and sample wells, **including sample background control wells**.
3. Mix well. Incubate for 5 minutes at 37 °C, protected from light.

Development solution

1. Immediately prior to use, dilute the 10× Development Solution 10-fold in ddH_2O to prepare a 1× development working solution, according to Table 4. 25 µL of the 1× development working solution is required for each reaction (well). Multiply the volumes in Table 4 according to the number of wells in the assay.

Table 4. Preparation of 1× development working solution, per one well*

10× Development Solution volume	ddH_2O volume	1× Development working solution final volume
2.5 µL	22.5 µL	25 µL

* Protect from light

2. Add 25 µL of the 1× development working solution to each of the standard and sample wells, **including sample background control wells**.
3. Mix well. Incubate for 10-15 minutes at 37 °C with gentle shaking, protected from light.

Measurement

Measure fluorescence intensity at:

λ_{ex} = 375 nm / λ_{em} = 465 nm

Results

Calculations

- An Excel-based calculation sheet is available at the Product Detail Page. **Use this sheet to calculate the test results**.
- If the Excel-based calculation sheet at the Product Detail Page is not used, calculations should be performed as follows:

1. Subtract the blank value (no standard) from all standards values
2. Plot the fluorescence measured for each standard against the standard amount per well.

Determine the linear regression equation. Use the following equation to calculate the collagen amount in the sample:

µg collagen = {[(Sample) – (Sample background control)] / Slope} × DF

Where:

Sample = fluorescence intensity units of the sample

Sample background control = fluorescence intensity units of the sample background control (no Digest Enzyme)

Slope = The standard curve slope, obtained from the linear regression equation

DF = Sample Dilution Factor (if sample is not diluted, the DF value is 1)

Note: If the sample was neutralized (see "Sample preparation" above), then a 2-fold dilution should be accounted for, in addition to any dilution of the sample, if applicable.

Example:

For 100 mg of tissue in 1 mL acetic acid, plus 1 mL of NaOH, for a total volume of 2 mL, 10 µL (0.5 mg tissue) was assayed using the kit.

- Mean sample = 16,588 RFU
- Mean sample background control = 761 RFU
- Slope = 10,689
- DF = 2 (to obtain the result in values of µg collagen per mg tissue)
- [(16,588 – 761) / 10,689] × 2 = 2.96 µg collagen per mg tissue

References

1. Tanzer, M.L., *Science*, **180(4086)**, 561-566 (1973).
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3. Kruegel, J., and Miosge, N., *Cell. Mol. Life Sci.*, **67(17)**, 2879-2895 (2010).
4. Khoshnoodi, J. *et al*., *Microsc. Res. Tech.*, **71(5)**, 357-370 (2008).
5. Feng, D. *et al*., *J. Biol. Chem*., **295(39)**, 13640-13650 (2020).

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