Ver.251101

Soluble Collagen Quantification Assay Kit

OSC1016-01 (100Tests)

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Product Description

Soluble Collagen Assay Kit detects newly synthesized (acid-soluble) collagen levels in tissues and cultured cell medium. The assay involves extraction of soluble collagen in acetic acid, followed by enzymatic degradation of collagen into glycine-rich oligopeptides, which are quantified using a fluorogenic reagent and developer solution that selectively react with the N-terminal glycine fragments to form a stable fluorescent complex (Ex/Em = 376/468 nm). The assay is more sensitive and selective than dye-binding (Picrosirius Red) soluble collagen assays, is simple to perform and has a linear range from 0.05–2µg collagen per well (2.5µg/mL to 100µg/mL in a 20µL sample volume).

Kit components

Reagent	Volume	Storage
Peptide Labelling Reagent	1 x 1Vial	-20°C
Detection Reaction Buffer	1 x 10mL	-20°C
Collagenase Enzyme Mix	1 x 1 Vial	-20°C
Collagenase I Standard	1 x 100µL	-20°C
Collagen Assay Buffer	1 x 25mL	-20°C
Developer Solution IX	1 x 500µL	-20°C

Storage:

Store kit at -20°C in the dark immediately on receipt and check below storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted. Reconstituted components are stable for 2 months. Aliquot components in working volumes before storing at the recommended temperature. Avoid repeated freeze thaws of reagents.

Reagents and Equipment Required but Not Provided

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well white opaque plate with flat bottom
- Multi-well spectrophotometer
- 0.5M acetic acid and 0.5M sodium hydroxide (NaOH*)*

Precaution

- To avoid contamination, use clean laboratory wares. Use clean, dry disposable pipette tips for dispensing. Close reagent bottles immediately after use.
- Avoid direct exposure of reagent to light. Do not blow into the reagent bottles.

1. Reagent Preparation

Before using the kit, spin tubes and bring down all components to the bottom of tubes.

− Prepare only as much reagent as is needed on the day of the experiment.

1.1 Collagen Assay Buffer: Bring to room temperature before use.

1.2 Collagenase Enzyme Mix: Reconstitute with 220µL Collagen Assay Buffer, divide into aliquots and store at - 20°C. Avoid multiple freeze/thaw cycles. Use within 2 months after reconstitution.

1.3 Detection Reaction Buffer: Bring to room temperature before use.

1.4 Peptide Labeling Reagent: Reconstitute with 275µL distilled water. Divide into aliquots and store at 20°C, protected from light. Prior to use, warm solution to room temperature and vortex thoroughly. Use within 2 months after reconstitution.

1.5 Developer Solution IX: Divide into aliquots and store at -20°C, protected from light. Prior to use, warm solution to room temperature and vortex thoroughly. Use within 2 months after opening.

1.6 Collagen I Standard : Provided as a 2 mg/mL stock solution of solubilized Type I collagen from rat tail tendon in dilute acetic acid. Store at 4°C and allow solution to come to room temperature prior to use.

2. Standard Preparation

- Always prepare a fresh set of standards for every use. Prepare a 0.2 mg/mL collagen solution by adding 20µL of the 2 mg/mL Collagen I Standard to 180µL of distilled water.
- Add 0, 2, 4, 6, 8, and 10µL of the 0.2 mg/mL working solution into a series of wells, generating 0, 0.4, 0.8, 1.2, 1.6 and 2µg of collagen/well.
- Adjust the volume of all standard wells (including the 0µg/well reagent blank) to 80µL/well with Collagen Assay Buffer.

Standard	0.2 mg/mL working solution (µL)	Collagen Assay Buffer (µL)	collagen/well (µg)
1	0	80	0
2	2	78	0.4
3	4	76	0.8
4	6	74	1.2
5	8	72	1.6
6	10	70	2.0

3. Sample Preparation

3.1 Soft Tissues and Adherent Cultured Cells (Acid Soluble Collagen):

For soft tissues:

- Rinse tissue samples with ice-cold distilled water or PBS to remove any residual blood, blot dry and mince with clean scissors. Transfer minced tissue to a pre-chilled glass bead homogenizer and add 1mL of ice-cold 0.5M acetic acid per ~100mg of wet tissue. Homogenize tissue on ice, transfer tissue homogenate to a microfuge tube, vortex thoroughly and incubate at 4°C overnight with gentle stirring/rotation (maximal solubilization of collagen is achieved after ~8 hours).

For adherent cells:

- Remove culture medium, detach cells manually using a rubber cell scraper and collect harvested cells in PBS. Pellet cells by centrifugation and aspirate PBS. Resuspend cell pellet in 1 mL of ice-cold 0.5 M acetic acid per ~1 x 107 cells. Transfer cell slurry to a pre-chilled Dounce homogenizer and homogenize on ice. Transfer homogenate to a microfuge tube, vortex thoroughly and incubate at 4°C overnight gentle stirring/rotation (maximal solubilization of collagen is achieved after ~8 hours).
- Following overnight incubation, centrifuge the homogenate at 10000 x *g* for 15 minutes at 4°C and transfer the acidic supernatant to a new microfuge tube. Neutralize acidic sample extract by adding an equal volume of 0.5M NaOH to the supernatant (i.e. mix 500µL of acidic supernatant and 500µL of 0.5 M NaOH).
- Add 2-20µL of neutralized sample extract to desired well(s) in a white 96-well plate. For each test sample, prepare two parallel sample wells, with one well serving as a sample background control. Adjust the volume of all wells to 80µL/well with Collagen Assay Buffer.

3.2 For cell culture medium (secreted soluble collagen):

- Collagen secreted into cell culture medium may be assayed directly, without the need for acid solubilization. Remove a sample of culture medium without detaching cells, centrifuge at 10000 x *g* for 15 minutes at 4°C to pellet any debris and transfer the clarified supernatant to a new microfuge tube. Add 10-20µL of clarified medium to two parallel wells (one well will serve as a sample background control) and adjust the volume to 80µL/well with Collagen Assay Buffer.

Note:

- **It is important to keep samples chilled during the homogenization procedure. Heat generated by homogenization can cause denaturation and crosslinking of soluble tropocollagen fibrils, rendering them insoluble in acid solutions.**
- **Soluble collagen levels and the effectiveness of acid solubilization can vary tremendously between tissues. Collagen present in "tough" tissues (such as cartilage or connective tissue) is highly crosslinked and tends to be resistant to acid solubilization.**

4. Assay Procedure

4.1 Prepare a working solution of collagenase by diluting the reconstituted Collagenase Enzyme Mix stock solution at 1:10 ratio with Collagen Assay Buffer. Prepare enough of the working solution to add 20µL to each reaction well (for 10 reactions, mix 20 µL of Collagenase Enzyme Mix stock and 180µL of Collagen Assay Buffer). Add 20 µL of collagenase working solution to test sample and standard wells. For sample background control wells, add 20 µL of Collagen Assay Buffer only. Incubate plate at 37°C for 60 minutes.

4.2 Following incubation, prepare detection reaction solution by diluting the reconstituted Peptide Labeling Reagent stock in Detection Reaction Buffer at a 1:30 ratio. Prepare enough of the working solution to add 75µL to each reaction well (for 10 reactions, mix 25µL of Peptide Labeling Reagent stock and 725µL of Detection Reaction Buffer). Add 75µL of detection reaction solution to all test sample and standard wells (including sample background control wells) and incubate plate (protected from light) at 37°C for 5 minutes.

4.3 Prepare developer working solution by diluting the Developer Solution IX stock in distilled water at a 1:10 ratio. Prepare enough diluted developer solution to add 25µL to each reaction well (for 10 reactions, mix 25µL of Developer Solution IX stock and 225µL of distilled water). Add 25µL of detection reaction solution to all test sample and standard wells (including sample background control wells). Incubate the plate (protected from light) at 37°C for 15 minutes with gentle orbital shaking to ensure well contents are effectively mixed.

4.4 Measure the fluorescence (Ex/Em = 376/468 nm) of all test sample and standard curve wells in endpoint mode.

5. Data Analysis

5.1 For the collagen standard curve, subtract the reagent blank (0µg/well collagen standard) fluorescence (RFU) value from all standard readings, plot the background-subtracted values and calculate the slope of the standard curve.

5.2 For test samples, subtract the corresponding sample background control well RFU value from the sample reading ($F = RFU_{Sample} - RFU_{BC}$) and apply the background-subtracted fluorescence (F) to the standard curve to get B µg of soluble collagen in the well.

Sample [soluble Collagen] = B/V x D = µg/µL

Where:

B is the amount of collagen, calculated from Standard Curve (µg).
V is sample volume added into the reaction well (µL)
D is sample dilution factor (D=1 when samples are undiluted)

Note: The calculation above gives the amount of collagen in the sample added to the well. The dilution factor D is only needed if the sample is diluted after the neutralization step. When calculating the amount of collagen in the original sample homogenate, remember to account for the 2-fold dilution that occurs during neutralization of the acidic homogenate.