

Version 3b Last updated 23 June 2025

# **ab241015**

# **Soluble Collagen**

# **Assay Kit**

For the detection of soluble Collagen in various biological samples and cultured cells.

This product is for research use only and is not intended for diagnostic use.

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## 1. Overview

Soluble Collagen Assay Kit (ab241015) 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 – 2 µg collagen per well (2.5 µg/mL to 100 µg/mL in a 20 µL sample volume).

## 2. Protocol Summary

Prepare tissue or cell samples and background controls.



Prepare standard curve.



Prepare collagenase working solution and add to standards, positive control and sample wells.



Measure fluorescence (Ex/Em = 376/468 nm) in endpoint mode.

### **3. General guidelines, precautions, and troubleshooting**

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:  
[www.abcam.com/assaykitguidelines](http://www.abcam.com/assaykitguidelines)
- For typical data produced using the assay, please see the assay kit datasheet on our website.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions

## 4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

<b>Item</b>	<b>Quantity</b>	<b>Storage condition</b>
Collagen Assay Buffer	25 mL	-20°C
Collagenase Enzyme Mix	1 vial	-20°C
Detection Reaction Buffer	10 mL	-20°C
Peptide Labeling Reagent	1 vial	-20°C
Developer Solution IX	500 µL	-20°C
Collagen I Standard II	0.1 mL	-20°C

## 5. Materials Required, Not Supplied

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
- 0.5 M acetic acid and 0.5 M sodium hydroxide (NaOH)

## 6. 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.

### 6.1 Collagen Assay Buffer:

Bring to room temperature before use.

### 6.2 Collagenase Enzyme Mix:

Reconstitute with 220  $\mu$ L Collagen Assay Buffer, divide into aliquots and store at  $-20^{\circ}\text{C}$ . Avoid multiple freeze/thaw cycles. Use within 2 months after reconstitution.

### 6.3 Detection Reaction Buffer:

Bring to room temperature before use.

### 6.4 Peptide Labeling Reagent:

Reconstitute with 275  $\mu$ L ddH<sub>2</sub>O. Divide into aliquots and store at  $-20^{\circ}\text{C}$ , protected from light. Prior to use, warm solution to room temperature and vortex thoroughly. Use within 2 months after reconstitution.

### 6.5 Developer Solution IX:

Divide into aliquots and store at  $-20^{\circ}\text{C}$ , protected from light. Prior to use, warm solution to room temperature and vortex thoroughly. Use within 2 months after opening.

### 6.6 Collagen I Standard II:

Provided as a 2 mg/mL stock solution of solubilized Type I collagen from rat tail tendon in dilute acetic acid. Store at  $+4^{\circ}\text{C}$  and allow solution to come to room temperature prior to use.

## 7. Standard Preparation

– Always prepare a fresh set of standards for every use.

**7.1** Prepare a 0.2 mg/mL collagen solution by adding 20  $\mu$ L of the 2 mg/mL Collagen I Standard II to 180  $\mu$ L of ddH<sub>2</sub>O.

**7.2** Add 0, 2, 4, 6, 8, and 10  $\mu$ L of the 0.2 mg/mL working solution into a series of wells, generating 0, 0.4, 0.8, 1.2, 1.6 and 2  $\mu$ g of collagen/well.

**7.3** Adjust the volume of all standard wells (including the 0  $\mu$ g/well reagent blank) to 80  $\mu$ L/well with Collagen Assay Buffer.

Standard #	0.2 mg/mL working solution ( $\mu$ L)	Collagen Assay Buffer ( $\mu$ L)	$\mu$ g collagen/well
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

## 8. Sample Preparation

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

#### For soft tissues:

**8.1** Rinse tissue samples with ice-cold ddH<sub>2</sub>O or PBS to remove any residual blood, blot dry and mince with clean scissors. Transfer minced tissue to a pre-chilled glass bead (Dounce) homogenizer and add 1 mL of ice-cold 0.5 M acetic acid per ~100 mg 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:

**8.2** 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 10<sup>7</sup> 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).

**8.3** Following overnight incubation, centrifuge the homogenate at 10000 x g for 15 min at 4°C and transfer the acidic supernatant to a new microfuge tube. Neutralize acidic sample extract by adding an equal volume of 0.5 M NaOH to the supernatant (i.e. mix 500 µL of acidic supernatant and 500 µL of 0.5 M NaOH).

**8.4** Add 2-20 µL 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 80 µL/well with Collagen Assay Buffer.

#### For cell culture medium (secreted soluble collagen):

**8.5** 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 min at 4°C to pellet any debris and transfer the clarified supernatant to a new microfuge tube. Add 10-20 µL of clarified medium to two parallel wells (one well will

serve as a sample background control) and adjust the volume to 80  $\mu$ L/well with Collagen Assay Buffer.

**Δ 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.

## 9. Assay Procedure

- 9.1 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 20  $\mu\text{L}$  to each reaction well (for 10 reactions, mix 20  $\mu\text{L}$  of Collagenase Enzyme Mix stock and 180  $\mu\text{L}$  of Collagen Assay Buffer). Add 20  $\mu\text{L}$  of collagenase working solution to test sample and standard wells. For sample background control wells, add 20  $\mu\text{L}$  of Collagen Assay Buffer only. Incubate plate at 37°C for 60 min.
- 9.2 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 75  $\mu\text{L}$  to each reaction well (for 10 reactions, mix 25  $\mu\text{L}$  of Peptide Labeling Reagent stock and 725  $\mu\text{L}$  of Detection Reaction Buffer). Add 75  $\mu\text{L}$  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 min.
- 9.3 Prepare developer working solution by diluting the Developer Solution IX stock in ddH<sub>2</sub>O at a 1:10 ratio. Prepare enough diluted developer solution to add 25  $\mu\text{L}$  to each reaction well (for 10 reactions, mix 25  $\mu\text{L}$  of Developer Solution IX stock and 225  $\mu\text{L}$  of ddH<sub>2</sub>O). Add 25  $\mu\text{L}$  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 min with gentle orbital shaking to ensure well contents are effectively mixed.
- 9.4 Measure the fluorescence (Ex/Em = 376/468 nm) of all test sample and standard curve wells in endpoint mode.

## 10. Data Analysis

- 10.1** For the collagen standard curve, subtract the reagent blank (0 µg/well collagen standard) fluorescence (RFU) value from all standard readings, plot the background-subtracted values and calculate the slope of the standard curve.
- 10.2** For test samples, subtract the corresponding sample background control well RFU value from the sample reading ( $F = \text{RFU}_{\text{sample}} - \text{RFU}_{\text{BC}}$ ) and apply the background-subtracted fluorescence (F) to the standard curve to get B µg of soluble collagen in the well.

$$\text{Sample [soluble Collagen]} = \mathbf{B/V \times D} = \mu\text{g}/\mu\text{L}$$

### 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)

**Δ 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.

## 11. Typical Data

Typical data provided for demonstration purposes only.

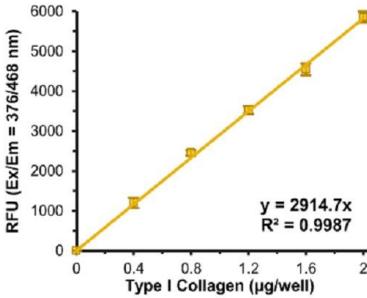


Figure 1. Collagen I Standard Curve.

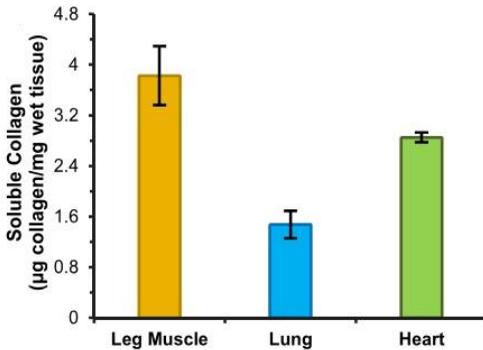
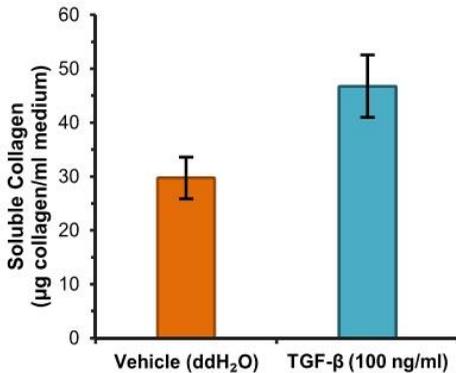


Figure 2. Estimation of acid-soluble collagen content in rat tissues. Rat leg muscle, lung and heart samples were homogenized in 0.5 M acetic acid, incubated overnight at 4°C to solubilize collagen and neutralized with 0.5 M NaOH. Collagen levels (calculated as µg collagen/mg wet tissue) for the samples were: 3.83 ± 0.47 µg/mg for muscle, 1.47 ± 0.22 µg/mg for lung and 2.85 ± 0.08 µg/mg for heart.



**Figure 3.** Estimation of secreted soluble collagen in cultured cell growth medium (DMEM/F12 medium). 3T3-L1 fibroblasts were grown to ~80% confluence in T-75 flasks and then treated with either vehicle or TGF-β (100 ng/ml), a cytokine known to stimulate synthesis and secretion of tropocollagen fibrils. After 48 hours of treatment, the culture medium was removed, centrifuged to remove debris and was assayed directly (each 10 µl clarified medium, undiluted). TGF-β treatment resulted in a roughly 1.5-fold increase in collagen secretion. Data are mean ± SEM of 3 replicates, assayed according to the kit protocol.

## 13. Notes

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