Soluble Collagen Assay Kit

Soluble Collagen Assay Kit is a detection kit for the quantification of soluble or solubilized collagens in tissue culture supernatants, cellular extracts and tissue homogenates.

Catalog number: ARG82627

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Collagen is the main structural protein in the extracellular matrix found in the body's various connective tissues. As the main component of connective tissue, it is the most abundant protein in mammals, making up from 25% to 35% of the whole-body protein content. Collagen consists of amino acids bound together to form a triple helix of elongated fibril known as a collagen helix. It is mostly found in connective tissue such as cartilage, bones, tendons, ligaments, and skin.

Depending upon the degree of mineralization, collagen tissues may be rigid (bone) or compliant (tendon) or have a gradient from rigid to compliant (cartilage). Collagen is also abundant in corneas, blood vessels, the gut, intervertebral discs, and the dentin in teeth. In muscle tissue, it serves as a major component of the endomysium. Collagen constitutes one to two percent of muscle tissue and accounts for 6% of the weight of strong, tendinous muscles. The fibroblast is the most common cell that creates collagen. Gelatin, which is used in food and industry, is collagen that has been irreversibly hydrolyzed. Collagen has many medical uses in treating complications of the bones and skin. [Provide by Wikipedia: Collagen]

PRINCIPLE OF THE ASSAY

This Soluble Collagen Assay Kit is a simple assay that measures the amount of soluble collagen or collagen that is solubilized by acetic acid alone or in combination with pepsin in biological samples such as tissue culture supernatants, cellular extracts and tissue homogenates. This assay is based on binding of the dye Sirius Red to collagen. Following binding of the dye, the collagen-dye complex precipitates, resulting in a coloured pellet. This colour can be released in an alkaline solution.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all reagent at 4°C upon receiving. Do not use kit components past kit expiration data.

Component	Quantity	Storage information
Assay Plate Set (1X V-shaped plate and 1X flat bottom plate)	1 ea	4°C
Balance Plate Set (1X V-shaped plate and 1X flat bottom plate)	1 ea	4°C
Standard (714 µg/mL in 20 mM acetic acid)	500 µL	4°C
Dilution Buffer	20 mL	4°C
Dye Solution (Sirius Red dye)	8 mL	4°C
Detection Solution (sterile alkaline buffer)	18 mL	4°C
Washing Buffer	80 mL	4°C
Plate Sealer	1 ea	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 540 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Microplate centrifuge (~3000xg)
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- It is important to perform the standard preparation and the sample preparation on ice and step 1-8 of the ASSAY PROCEDURE below 25°C. If the temperature in the laboratory is higher than 25 °C, these steps should also be performed on ice. This is because of the stability of the collagen in samples and standards.
- Thoroughly mixing of sample and Dye Solution is important. This is done by multiple pipetting up and down. An end-over-end roller can also be used. Do not forget to seal the plate carefully.
- Centrifugation must be carried out in a refrigerated centrifuge at a temperature below 25°C, to prevent denaturation of collagen.
- Removal of the supernatant following centrifugation should be performed very carefully in order to prevent pellets from falling out. Generally the following methods give good results. Put the plate at a 45° angle on a stack of paper towels. If the solution doesn't flow out spontaneously, gently

move the plate. If flow starts keep the plate upside down on the towels for a while. Not all the supernatant has to be removed; if some liquid is left this will be removed during the washing step.

- Some sample types give pellets which fall out easily. If this occurs use a multichannel pipet to remove the liquid and be careful not to disturb the pellet. For safety do not try to remove all liquid.
- If Washing Buffer is added using a multichannel pipet, please be careful not to disturb the pellet with the liquid flow in order to prevent loss of the pellet. Generally manual multichannel pipettes give the best results.
- Depending on the sample type pellets could be easily lost during washing. If this occurs use a multichannel pipet to remove the liquid and be careful not to disturb the pellet.
- Serum present in the samples does not affect the assay up to a concentration of at least 10% (v/v) serum.
- If the centrifuge cannot reach 3000 x g, use longer centrifugation times.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell culture medium: centrifuged at 1,500 x g for 10 minutes at 4°C to remove cell debris. The supernatant is tested either undiluted or diluted to a proper range using Dilution Buffer or non-conditioned culture medium (if the culture medium contains serum). Up to 10% (v/v) serum does not affect the assay.

Cellular extracts: The medium on the cells is removed, and the cells are washed with 1X PBS. Replace the PBS by 0.5 M Acetic acid (250 µl/well of 24-well-plate), incubate at 4°C for overnight on a rotating platform, and make a cellular extract using a rubber policeman. The extract is centrifuged for 10 minutes at 3,000 x g, and the supernatant is tested in the assay in 1-10 fold dilutions made in Dilution Buffer.

Note:

- Serum present in the samples does not affect the assay up to a concentration of at least 10% (v/v) serum.
- It is important to keep the samples cold, preferably on ice, and always below 25°C. Do not thaw samples in a 37°C water bath, but use a 25°C water bath. Collagen can denature at higher temperatures and denatured collagen is not detected in the assay

REAGENT PREPARATION

- **Standard:** Prepare all standards on ice. The Standard has a concentration of 714 µg/mL (in 20 mM acetic acid). This stock is diluted 10-fold in Dilution Buffer (E.g., add 120 µL of Standard to 1080 µL of Dilution Buffer, resulting in 1200 µL of the 10-fold dilluted collagen stock (71.4 µg/mL). Store the rest of standard stock at 4°C for up to 1 month.

Standard tube	Collagen/well (µg)	Dilution Buffer (µL)	10-fold diluted Standard, 71.4 µg/mL (µL)
S1	10	0	350
S2	8	70	280
S3	6	140	210
S4	4	210	140
S5	2	280	70
S6	1	315	35
S7	0.5	332.5	17.5
S0	0	350	0

Note: It is important to perform this procedure on ice.

- **Dye Solution**: Ready to use. If opened, store at 4°C for up to 1 month.
- **Dilution Buffer**: Ready to use. If opened, store at -20°C for up to 6 months.
- **Washing Buffer**: Ready to use. If opened, store at -20°C for up to 6 months.
- **Detection Solution**: Ready to use. If opened, store either at 4°C or room temperature for up to 1 month.

ASSAY PROCEDURE

It is recommended that all samples and standards be assayed in duplicate. It is important to perform step 1-8 below 25°C (Or keep the assay on ice.)

1. Pipette **140 µL** of **each diluted standard** into appropriate wells of the **assay microplate (V-shaped plate)**
2. Pipette **140 µL** of **Dilution Buffer** into appropriate wells for use as **blank**
3. Pipette **140 µL** of **samples** (or sample dilutions) into the appropriate wells
4. Add **60 µL** of **Dye Solution** to each well
5. Mix thoroughly by pipetting up and down **at least five times**. An end-over-end roller can also be used.
6. Seal the plate with the Plate Sealer present in kit.
7. Incubate **10 minutes** at **room temperature (<25°C), or on ice.**
8. Place the assay microplate (V-shaped plate) in the reading microplate (flat bottom plate). Centrifuge this set in a centrifuge equipped with a microplate swing out carrier for **60 minutes** at **3,000 x g** at **<25ºC**, preferably at **4°C**. Use a Balance Plate Set with 200 µL of distilled water per well for balance. (If the centrifuge cannot reach 3000 x g, use a longer centrifugation time.)
9. After centrifugation, carefully remove the seal.
10. ***Do not remove the supernatant,*** but carefully add **100 µL** of **Washing Buffer** to each well.
11. Remove supernatant and Washing Buffer (unbound dye) by placing the plate at a 45º angle on a (5 cm thick) stack of paper towels and carefully blotting the liquid in the wells by towels. If the solution doesn't easily flow out of the plate, gently move the plate. Not all solution has to get

out (remaining solution is removed during washing steps). Removing the supernatant can be difficult. The pellet (=collagen complex) must not be disturbed!

12. Carefully add **250 µL** of **Washing Buffer** to each well and remove this solution by placing the plate at a 45° angle on a stack of paper towels and blotting the liquid in the wells by towels as described above.

Note:

- Do not pipet up and down! Please be aware that the liquid flow is not disturbing the pellet.
- Wells might overflow during this first washing step, this has no influence on the performance of the assay.

13. For a second wash step repeat previous step.
14. Place the plate upside down on a paper towel and keep for **at least 5 minutes** to remove remainder of Washing Buffer by paper towels.
15. Add **150 µL** of **Detection Solution** to the pellets and mix thoroughly by pipetting up and down **at least ten times**.
16. Transfer **100 µL** of **the coloured solution** to the **reading microplate (flat bottom)**
17. Read the plate at **O.D. 540 nm** and perform data analysis.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard or sample. Subtract the average blank.
2. Create a standard curve by plotting the mean A_{540} of each standard on the y-axis against the collagen content on the x-axis (0, 0.5, 1.0, 2, 4, 6, 8, 10 µg collagen/well). Draw a best-fit linearized curve through the points on the graph.
3. Using this standard curve the A_{540} values of the test samples can be calculated to µg collagen/well. From the sample volume used in the assay the collagen concentrations can be calculated.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Soluble Collagen Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.