bio col or life science assays

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Sircol™ 2.0 Quickstart Guide

Sircol 2.0™ is designed for quantitative measurement of soluble collagen in material from *in-vivo* & *in-vitro* sources.

The kit employs colorimetric detection in a convenient microplate-based format.

First Steps

Plan: Use the **flowchart** on the back of this guide to select a suitable preparation protocol for your samples. Sections of the product manual that are indicated in the protocol should be read in combination with this guide.

Prepare: Ensure you have access to the necessary supporting equipment, indicated on **p6-7** of the product manual.

Proceed with the General Protocol...

A

General Protocol
(see page **9-10** of manual for further detail)

Setup of samples / standards / controls

Setup samples: Process samples according to the selected preparation protocol. We recommend assaying prepared samples in duplicate (as a minimum).

NB: Samples may be frozen (-20°C) for assay at a more convenient time.

Setup controls: It is always good practice to run assay controls. As a minimum, we would advise running a 'plate blank', comprising 200µl of Dye Release Reagent. This should be subtracted from all other readings to correct for microwell plate absorbance. You may also wish to run a 'reagent control', comprising sample extraction reagent. This is typically 0.5M Acetic Acid, cell culture medium, extraction buffer or deionised water.

Setup standards: Prepare the standards according to **Table.1.** These can be prepared directly within the microplate wells.

Table.1 Preparation of assay Standards

Standard Collagen Concentration (µg/ml)	Mass of Collagen per well (µg)	Volume of STANDARD to be added (µl)	Volume of DILUENT* to be added (µl)
0	0	0	100
10	1	5	95
50	5	25	75
100	10	50	50
150	15	75	25
200	20	100	0

**Diluent can be water or unused sample extraction reagent.*

Retain a map of the placement of samples, controls, and standards in the microplate!

B

Dye-labelling of collagen

1. Add prepared assay samples directly to the wells of the microplate. The maximum recommended sample volume per well is 100µl.
2. Then add 175µl of Sircol Dye Reagent to wells containing standard or sample (excluding the plate-blank). Apply a microwell plate seal (provided with the kit) to fully seal the wells of the plate, then reapply the lid.
3. Place microplate on a microplate shaker and shake for 30 min at 300 rpm. During this time any dye-labelled collagen will form an insoluble precipitate.

C

Collection and washing of dye-labelled collagen

4. Transfer the microplate to a suitable centrifuge and spin at 1500 x *g* for 90 min. If using a lower speed class centrifuge (such as those used for PCR plates) then a 400 x *g* spin for 120 min is sufficient. During this time any precipitated collagen is collected and retained on the plate base.

   NB: To aid subsequent liquid removal the plate(s) should be placed in the centrifuge so that Row H is positioned nearest the rotor. Do not exceed a maximum force of 2000 x g. Always use an equivalently weighted balance plate during each centrifuge step.

5. Remove plate from centrifuge and carefully remove the microwell plate seal. The total liquid contents of each well should be removed using **gentle(!)** aspiration:

   NB: Dye-labelled collagen will be present as a loosely attached deposit on the base and sidewalls of the wells. Be careful not to accidently remove this by excessive suction or scratching with the aspiration tip or needle.

   Helpful tip: We recommend manual aspiration via micropipette. The microwell plate should be held at a 45° angle and the pipette tip positioned against the base of each well, at the angle formed between microwell wall and base. The liquid may then be gently removed.

   If using a 96-well Aspirator, please ensure it is setup to avoid removing any precipitated material from the base of the wells.

6. Add 250µl of Plate Wash Reagent to each well (excluding the plate-blank). Then apply a microwell plate seal. Centrifuge the plate at a force of 1500 x *g* for 30 min (alternatively 400 x *g* for 60 min can be used).

D

Release of dye-label from the collagen precipitate

7. Carefully remove the liquid from all wells (using the same procedure as per point '5'). Then add 200µl of Dye Release Reagent to the appropriate sample/standard/blank microwells. Re-cover the plate with an adhesive Microwell plate seal.
8. Place the plate on the microplate shaker (700 rpm) for approximately 20-30 min until a uniform coloration is observed in the highest concentration assay standards.

   The microwell plate seal should be carefully removed, the assay microplate is now ready for measurement.

Turn to p10 of the product manual to continue with measurement & analysis.

Sircol 2.0 Sample preparation & analysis flowchart

Please use the flowchart to identify the most appropriate sample preparation protocol for your samples. Samples require preparation to ensure that collagen has been released in a soluble form, suitable for Sircol 2.0 analysis. *See **p18-19** of manual for more detailed explanation.*

Where has the sample material been derived from?

See **p18-19** of manual for useful information on samples & preparation. Species applicability information can be found on **p6**.

in-vivo sources?

Such as: Muscle, lung, liver, cartilage, tendons, aorta, valves, skin

*see **p20** of manual for more information*

- Prepare sample for extraction by dicing into small cubes (2-3 mm).
  (Rinse clean using cold PBS)
  *see preparation guidelines on **p21** of manual*
- Prepare sample for extraction by homogenising
  (Keep sample cool to avoid collagen denaturation)
  *see preparation guidelines on **p21** of manual*

EXTRACTION:
Release soluble collagens using OVERNIGHT ACID-PEPSIN DIGESTION (at +4˚C)
*Follow protocol on **p21** of manual*

in-vitro sources?

Such as 2D or 3D cell culture

*see **p23** of manual for more information*

- **Are you measuring soluble collagen in culture media?**
  - Prepare media for analysis
    *Follow protocol on **p24** of manual*
- **Are you measuring Matrix-associated collagen (from the ECM).**
  - ***EXTRACTION:***
    Release soluble collagens using OVERNIGHT ACID-PEPSIN DIGESTION (at +4˚C)
    - 2D cell culture
      *Follow protocol on **p24** of manual*
    - 3D cell culture
      *Follow protocol on **p25-26** of manual*

Perform ASSAY Follow General Protocol on **p9** of manual

Analyse Data

*see **p14** of manual*

Results!

- Is the sample collagen concentration ‹20µg/ml
  - Use Collagen Concentration Protocol
    *see **p11** of manual for more detail*
  - **Troubleshooting**
- Is the sample collagen concentration ›200µg/ml
  - Dilute samples before assay
    *see **p15** of manual for more detail*
  - **Troubleshooting**

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