

## **Sircol Insoluble<sup>™</sup>** **Insoluble Collagen Assay Kit**

The **Sircol<sup>™</sup> INSOLUBLE Collagen Assay** is a dye-binding method for analysis of insoluble collagen fibers.

**Standard Kit: S2000 (100 assays) Economy Pack: S6000 (400 assays)**

### **Kit Components**

- Fragmentation Reagent for insoluble collagen fibres. Contains dilute acetic acid, anti-microbial agents and surfactants.
- Dye Reagent contains Sirius Red formulated for specific binding to collagen under the conditions defined in the Sircol Manual.
- Alkali Reagent contains 0.5M sodium hydroxide and is used to recover Sircol Dye from the pellet of the collagen-dye complex.
- Reference Standard: 1000µg/ml. A sterile solution of cold, water-soluble denatured collagen from bovine skin. Stored in 0.1M acetic acid within a sealed vial.
- Acid-Salt Wash Reagent (Concentrate) – contains acetic acid, sodium chloride and surfactants.
- Screw capped round bottomed micro-centrifuge tubes, 2ml capacity.

### **Method Summary**

1. Native insoluble collagen is converted to soluble denatured collagen using the fragmentation reagent.
2. Test materials, standards and reagent blanks are mixed with Sircol Dye reagent in microcentrifuge tubes for 30 minutes. The collagen-dye complex formed precipitates out from the soluble unbound dye.
3. The complex is packed firmly to the bottom of the tubes by centrifugation at ~12,000rpm for 10 minutes. Unbound dye is removed by inverting and draining tubes.
4. Ice-cold acid-salt wash is gently added to remove unbound dye from pellet surface and microcentrifuge tube. The tubes are centrifuged at ~12,000rpm for 10 minutes and the wash drained off.
5. Bound dye is then released and dissolved via addition of alkali reagent and vortex mixing for 10 minutes.
6. Released dye is measured spectrophotometrically at 550nm or colorimetrically with a blue-green filter using a microplate reader.
7. Calculate to express results as native collagen.

## Frequently Asked Questions

What is the detection limit of the Insoluble Sircol assay? 10.0µg

How long does the Sircol assay take to run? 4 hours

How stable is the Sircol assay? Shelf life of 1 year at +10°C to 25°C unopened.  
Do not freeze kit or remove metal seal from collagen standard.

What forms of collagen does the Sircol assay measure? Insoluble collagen fibres.

What types of collagen can the Sircol assay be used with? Mammalian collagens, Types I, II, III, V & XI.

What absorbance values can samples be measured at? Absorbance spectrum maximum for Sircol dye in alkali reagent is 555nm however absorbance settings within the range 500-570nm will give suitable readings.

How to prepare samples

Tissue samples are exposed to dilute acid at a temperature of 65°C for not less than 2 hours and for not more than 3 hours. Under these conditions, the insoluble collagen fibres are converted to water-soluble denatured collagen, without generating the release of hydroxyproline residues.

Bone and calcified cartilage samples are treated with fragmentation reagent containing EDTA to decalcify.

**Technical Details** – the complete assay manual, free of charge, can be viewed and/or printed from our website at [www.biocolor.co.uk](http://www.biocolor.co.uk).

Biocolor Ltd.  
8 Meadowbank Road, Carrickfergus,  
Co. Antrim, N. Ireland, BT38 8YF

Telephone: 028 93350258  
Fax: 028 93369716  
Email: [info@biocolor.co.uk](mailto:info@biocolor.co.uk)  
[www.biocolor-assays.com](http://www.biocolor-assays.com)