

A Simple Quantitative Micro-Assay Kit for Collagen and Non-Collagenous Proteins

This kit provides a simple quantitative micro-assay tool for determining the amounts of collagen and non-collagenous proteins in cultured cell layers and tissue sections by differential staining with two dyes, Sirius Red and Fast Green (1-2). Sirius Red binds specifically to collagen, whereas Fast Green stains non-collagen proteins. This method has been applied for measurement of collagen contents in various tissues (3-7).

These dyes can be easily extracted from stained tissues using 0.05M NaOH in methanol, and measured at OD 540 (Sirius Red) and OD 605 (Fast Green), respectively. The amounts (μg) of collagen and non-collagenous proteins in each section can be calculated using simple equations. The assay sensitivity is high enough to determine collagen and non-collagenous proteins in tissue sections with 10-20 μm thick prepared for general histological studies. The assay sensitivity for collagen is more than 3 $\mu\text{g}/\text{section}$, and non-collagenous protein is more than 50 $\mu\text{g}/\text{section}$.

Reagents included

1. Dye solution: 10 ml of mixed dye solution (0.1 % Sirius Red and 0.1% Fast Green dissolved in water saturated with picric acid): For 30-50 samples. Store at RT up to 1 year.
NOTE: Saturated picric acid is important for blocking the non-specific binding of Sirius Red to non-collagenous proteins and DNA. Do not dilute the dye solution with water.
2. Dye extraction solution: 100 ml of a mixture of 0.1N NaOH and Methanol (1:1)

Procedure

Paraffin embedded tissue section

1. Prepare paraffin embedded tissue sections (approximately 30-50 μm^2 , 10-20 μm thick) according to standard method.
2. Deparaffinize the tissue section by Xylose, Xylose-Ethanol (1:1), Ethanol, Ethanol-Water (1:1) and then Water.
3. Transfer individual samples to Petri dishes.
4. Cover tissue slice on a slide glass with 0.2-0.3 ml of dye solution and incubate in a Petri dish at room temp for 30 min.
NOTE: To avoid evaporation of dye solution, add wet filter paper under the tissue slide and cover the Petri dish with a lid.
5. Carefully remove dye solution and rinse the tissue section with water until the fluid is colorless.
6. Add 1 ml of dye extraction solution and gently mix until the color is eluted from the tissue section.
7. Read the color at OD 540 and OD 605 by spectrophotometer.

Cell layer cultured in vitro

1. Place a sterilized round glass slide at the bottom of individual wells of 24-well culture plate if necessary.
2. Culture cells directly in 24 well culture plate or on the glass surfaces for desired period.
3. Remove culture medium and wash the plate or the glass slide with PBS.
4. Fix the cells with ethanol (add ethanol and incubate for 10 min at room temperature)
5. Then stain the cell layer with the dye solution and follow the procedure described above.

Calculation

To calculate the amounts of collagen, first, correct the OD 540 value by subtracting the contribution by Fast Green, which interfere in the absorbance at 540 nm. Fast Green contributes 29.1% of the OD 540 value. The Color equivalence (OD values/mg protein) is 37.8 for collagen and 2.04 for non-collagenous protein at OD 540 and 640, respectively (2).

$$\text{Collagen } (\mu\text{g/section}) = [\text{OD } 540 - (\text{OD } 605 \times 0.291)] / 37.8 \times 1000$$

$$\text{Non-collagen protein } (\mu\text{g/section}) = \text{OD } 605 \text{ values} / 2.04 \times 1000$$

Collagen types stained by Sirius Red

In general, collagen stained by Sirius Red has a fibrillar pattern, whereas non-collagen proteins stained by Fast Green have a more diffused pattern. Based on the staining patterns in different tissues, it is apparent that Type I, III, IV and V Collagen (7) can be stained as well as Type II Collagen, which is dominant component in cartilage. Since many types of collagen exist in tissues at variety of ratios and the color equivalence for each type of collagen has not determined, one color equivalent value is used for all types of collagen.

NOTE: Gel Staining

Sirius Red and Fast Green have been used to stain Gel such as SDS-gel and Isoelectric focusing gel for staining collagen and non-collagenous proteins, respectively. For example, 0.1% Sirius Red dissolved in saturated picric acid can be used for staining various types of collagen (7) in gels, and gels will be destained with MeOH-Acetic Acid-Water (30 : 7 : 63).

Fast green has an advantage for quantitative analysis of proteins on SDS-gel, because this dye remains on proteins without loss of stain during destaining process, whereas Coomassie blue significantly loses the stain during destaining process with MeOH-Acetic Acid-Water. Fast Green (0.25%) dissolved in 10% acetic acid has been used for staining non-collagenous proteins (8-9) in SDS-gels and isoelectric focusing gels. The gels is destained with MeOH-Acetic Acid-Water (30 : 10 : 60).

However, we do not recommend this mixed solution of Sirius Red and Fast Green for staining gel, because Sirius Red non-specifically binds to non-collagenous proteins and blocks the binding of Fast Green in gel.

References

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