

PICRO-SIRIUS RED STAIN KIT

DESCRIPTION

For identifying collagen fibers in paraffin-embedded tissue sections

Intended Use

This kit is intended for use by laboratory professionals to stain prepared paraffin embedded tissue specimens to identify collagen and muscle.

Principle and Results

Picrosirius red (PSR) staining is designed for collagen detection and quantitative estimation in histological sections of normal and abnormal tissues (Lopez De Padilla *et al.*, 2021; Segnani *et al.*, 2015). PSR is an anionic dye with sulfonic acid groups that react with basic amino acids in collagen molecules (Junqueira *et al.*, 1979). PSR also enhances the birefringence of collagen under polarization, since the PSR molecules align parallel to the long axis of each collagen fiber.

In bright-field microscopy, collagen is red on a pale-yellow background. Picrosirius red can cause appreciable de-staining of the nuclei.

Under polarized light, thick collagen type I fibers appear yellowish-orange, orange, to red, whereas thin collagen type III fibers appear green to yellowish green against a black background (Kiernan).

Specimen Collection

Samples should be appropriately fixed, paraffin-embedded 4-5 µm tissue section.

Kit Storage and Use

Store each kit component according to the recommended temperature and use before expiration on the label.

Precautions

See SDS for complete warnings, precautions, hazard and precautionary statements, and disposal information.

KIT CONTENTS

- 6259A Weigert Hematoxylin
- 6259B Weigert Ferric Chloride
- 6259C Picro-Sirius Red
- 6259D Acidified Water

PROCEDURE

Before You Begin

Prepare Weigert's working solution by combining Weigert Hematoxylin and Weigert Ferric Chloride into a 1:1 ratio. Mix well.

Autostainer Configuration

This stain kit may be adapted for use on most open platform autostainers. Sixteen baths are needed to run the complete procedure. A minimum of five baths is required to perform this procedure excluding the deparaffinization, hydrations, dehydrations, and clearing steps.

#	ACTION	SOLUTION	HEAT	TIME		DETAILS
				MIN	SEC	
1	DEPARAFFINIZE	XYLENE OR SUB 2 CHANGES	—	5	—	5 MINUTES EACH CHANGE OR AS REQUIRED
2	HYDRATE	GRADED ALCOHOLS	—	1	—	1 MINUTE EACH CHANGE OR 10 DIPS PER GRADED ALCOHOL
3	RINSE	RUNING TAP WATER	—	1	—	
4	IMMERSE	WORKING WEIGERT'S SOLUTION	—	10	—	
5	RINSE	RUNNING TAP WATER	—	10	—	
6	IMMERSE	PICRO-SIRIUS RED	—	60	—	INCREASING TIME DOES NOT AFFECT STAIN. DO NOT SHORTEN STAINING TIME.
7	WASH	ACIDIFIED WATER 2 CHANGES	—	—	5	5 SECONDS EACH CHANGE. PHYSICALLY REMOVE MOST OF THE WATER BY VIGOROUSLY SHAKING OR BLOTING WITH DAMP FILTER PAPER.
8	DEHYDRATE	ABSOLUTE ALCOHOL 2 CHANGES	—	—	—	10 DIPS PER CHANGE OF ABSOLUTE ALCOHOL
9	CLEAR	XYLENE OR SUB 2 CHANGES	—	5	—	5 MINUTES EACH CHANGE OR AS REQUIRED
10	COVERSLIP	RESINOUS MEDIUM	—	—	—	

Notes

1. Type 4 collagen in basement membranes, keratohyalin granules, and some types of mucus are stained red but are not birefringent membranes. It is necessary to rotate the slide to see all the fibers, in any single orientation the birefringence of some fibers will be extinguished.
2. Formalin-fixed tissue will show higher histological quality compared to Bouin-fixed tissues.
3. Collagen bundles usually appear crimped or wavy with alternating transverse dark bands under polarized light microscopy. It is said that dark collagen bands are at extinction. Studies have shown that 45° with respect to crossed polarizers provide the best qualitative and quantitative assessment of collagen.
4. Some permanent Mounting Mediums may cause fading over time with picric acid-based stains. Be sure to test your preferred Mounting Medium.

REFERENCES

1. Junqueira, LC, Bignolas, G, & Brentani, RR. (1979). Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J*, 11(4), 447-455. <https://doi.org/10.1007/BF01002772>
2. Kiernan, JA. *Sirius red for collagen staining protocol*. Dept Anatomy & Cell Biology The Univ. of Western Ontario
3. Lopez De Padilla, CM, Coenen, MJ, Tovar, A, De la Vega, RE, Evans, CH, & Muller, SA. (2021). Picrosirius red staining: Revisiting its application to the qualitative and quantitative assessment of collagen type I and type III in tendon. *J Histochem Cytochem*, 69(10), 633-643. <https://doi.org/10.1369/00221554211046777>
4. Segnani, C, Ippolito, C, Antonioli, L, Pellegrini, C, Blandizzi, C, Dolfi, A, & Bernardini, N. (2015). Histochemical detection of collagen fibers by sirius red/fast green is more sensitive than van Gieson or sirius red alone in normal and inflamed rat colon. *PLoS One*, 10(12), e0144630. <https://doi.org/10.1371/journal.pone.0144630>

PRODUCT INFORMATION

CAT. #	DESCRIPTION
6259A	Weigert Hematoxylin, 16oz
6259B	Weigert Ferric Chloride, 16oz
6259C	Picro-Sirius Red, 16oz
6259D	Acidified Water, 16oz

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