BIOGNOST®

PICRO-SIRIUS RED KIT

IVD *In vitro* diagnostic medical device CE

Three-reagent kit for collagen visualization

INSTRUCTIONS FOR USE

REF Product code: PSR-100T (for 100 tests)

Introduction

Picro-Sirius Red kit is used for visualization and collagen staining of tissues fixed in formalin and embedded in paraffin. It contains Sirius Red dye used in histology for demonstrating collagen fibers. Strong linear anionic dye stains collagen at low pH by reacting with base groups on collagen molecules. Sirius Red parallelly binds to collagen molecule; it also enhances collagen birefringence so it can be easily identified by observing the section under polarized light. Kit contains Eriochrome cyanine R dye that counterstains nuclei grey-brown. Eriochrome is acid-fast; that prevents nuclei from being discolored after longer incubation in Picro-Sirius Red solution.

Product description

- **PICRO-SIRIUS RED KIT** - Kit used for collagen staining and differentiation in histology.

The kit contains:	100 tests (PSR-100T)
ErioGnost reagent	ERS-OT-30 mL
Picro-Sirius Red reagent	PSR-OT-30 (30 mL)
Acetic acid, 0.5% solution	OK05-OT-100 mL (100 mL)
Plastic Pasteur pipette	1 pc

Other sections and reagents that may be used in staining:

- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 95 and Histanol 100
- Clearing agents, such as BioClear xylene or a substitute, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus, BioWax 56/68, BioWax Blue, BioWax Micro.
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's VitroGnost glass slides
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, and BioMount C
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm

Preparing the histological sections for staining

- Fix the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax Plus, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to **4-6** μm slices and place them on a VitroGnost glass slide.

NOTE

Apply the reagent so it completely covers the section.

Histological sections staining procedure

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Add ErioGnsot reagent (for special staining kits) (≥5 drops)	5 min
6.	Rinse with tap water	4 min
7.	Add Picro-Sirius Red reagent (≥5 drops) Note: use incubation tray in order to prevent evaporation	60 min
8.	Use a Pasteur pipette to add and rinse the section using Acetic acid, 0.5% solution Physically remove the solution from the glass slide by shaking	2 exchanges
9.	Dehydrate using 100% alcohol (Histanol 100)	3 exchanges, 1 min each
10.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with VitroGnost cover glass.

Result

Collagen - red
Nuclei - grey-brown
Rest of tissues - pink-red

Under polarized light:
Thick collagen fibers - orange or yellow
Thin collagen fibers - green

Note

Time periods of staining processes are not entirely standardized and they approximately correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

Preparing the sample and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples with modern technology and mark them clearly. Follow the manufacturer's instructions for handling. In order to avoid mistakes, the staining procedure and diagnostics should only be conducted by authorized and qualified personnel. Use only microscope according to standards of the medical diagnostic laboratory.

Safety at work and environmental protection

Handle the product in accordance with safety at work and environmental protection guidelines. Used solutions and out of date solutions should be disposed of as special waste in accordance with national guidelines. Chemicals used in this procedure could pose danger to human health. Tested tissue specimens are potentially infectious. Necessary safety measures for protecting human health should be taken in accordance with good laboratory practice. Act in accordance with signs and warnings notices printed on the product's label, as well as in BioGnost's material safety data sheet.

Storing, stability and expiry date

Keep Picro-Sirius Red kit in a tightly closed original package at temperature of +15 to +25°C. Do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Rittié L. (2017) Fibrosis. Methods in Molecular Biology, vol 1627. Humana Press, New York, NY, pp 395-407.
2. Carson, F. L., Hladik, C. (2009): Histotechnology: A Self-Instructional Text, 3rd ed., Chicago: ASCP Press

PSR-100T, V1 EN1, 12 January 2021, NM/IŠP

	Refer to the supplied documentation	°C	Storage temperature range		Number of tests in package	REF	Product code	CE	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	CE
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until	LOT	Lot number		Manufacturer		
IVD	For *in vitro* diagnostic use only		Keep in dry place		Caution - fragile						