BIOGNOST®

SAFRANIN O KIT

IVD *In vitro* diagnostic medical device CE

Four reagent kit for identification of cartilage, mucins, and mastocyte granules used in histology and cytology

INSTRUCTIONS FOR USE

REF Product code: SAFO-100T (for 100 tests) SAFO-K-100 (4 x 100 mL)

Introduction

Safranin O kit is used for visualizing and staining of cartilage, mucins and mastocytes in formalin fixed and paraffin mounted tissue samples, but also in frozen tissue sections. It contains Safranin O dye, used in histology and cytology as red nuclear counterstain. Safranin O dye is often used for detecting chondrocytes originating from human and rodent mesenchymal stem cells. Fast Green F.C.F. dye stains background structures, creating a clear and visually rich contrast. Optionally, ErioGnost reagent can be used to achieve selective nuclear staining with a darker hue than with using Safranin O reagent.

Product description

SAFRANIN O KIT - Kit for staining cartilage, mucins and mastocytes.

The kit contains:	100 tests (SAFO-100T)	4 x 100 mL (SAFO-K-100)
ErioGnost reagent	30 mL (ERS-OT-30)	100 mL (ERS-OT-100)
Fast Green F.C.F. contrast reagent	30 mL (FGKR-OT-30)	100 mL (FGKR-OT-100)
Acetic acid, 1% solution	30 mL (OK1-OT-30)	100 mL (OK1-OT-100)
Safranin O reagent	30 mL (SAFO-OT-30)	100 mL (SAFO-OT-100)

Other reagents and that may be used with the method:

- 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 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and embedding media, such as BioGnost's granulated paraffin BioWax Plus 56/58, BioWax 56/68, BioWax Blue, BioWax Micro.
- Glass slides used in histology, pathology and cytology, such as VitroGnost SUPER GRADE or VitroGnost COLOR , or one of 30 (and more) BioGnost's 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
- BioGnost's immersion oils, such as Immersion oil and Immersion oils types A and C

Preparing histological sections for staining

- Fix the tissue sample thoroughly (4% NB Formaldehyde, 10% NB Formaldehyde), 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 embed the sample in paraffin (BioWax Plus 56/58, 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.

Sample staining procedure

a) using kit for 100 tests (SAFO-100T)

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 exchanges, 2 min each
4.	Rehydrate in distilled (demi) water	2 min
5.*	Stain using ErioGnost reagent (add ≥5 drops)	5 min
6.*	Rinse under tap water	4 min
7.	Stain using Fast Green F.C.F. counterstain reagent (≥5 drops)	2 min
8.	Rinse in distilled (demi) water Note: this step may be prolonged in case of weaker counterstaining	10 seconds
9.	Treat with Acetic acid, 1% solution (add ≥5 drops)	10 seconds
10.	Stain with Safranin O reagent (add ≥5 drops)	5 min
11.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 2 min each
12.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 2 min each
13.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

* If darker nuclear staining is not necessary, these steps can be skipped.

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, apply 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.

b) using kit with four 100 ml reagents (SAFO-K-100)

Pour the reagents into glass staining jars (Coplin, Hellendahl or Schifferdecker), return to original bottles after staining. Close tightly. Filter the reagents if necessary.

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 exchanges, 2 min each
4.	Rehydrate in distilled (demi) water	2 min
5.*	Immerse in ErioGnost reagent	5 min
6.*	Rinse under tap water	4 min
7.	Immerse into Fast Green F.C.F. contrast reagent	2 min
8.	Rinse in distilled (demi) water Note: this step may be prolonged in case of weaker counterstaining	10 seconds
9.	Immerse into Acetic acid, 1% solution	10 seconds
10.	Without rinsing, immerse into Safranin O reagent	5 min
11.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 2 min each
12.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 2 min each
13.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

* If darker nuclear staining is not necessary, these steps can be skipped

Immediately after clearing, apply an appropriate BioMount covering/mounting medium on the section. If BioClear xylene was used, apply 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

Nuclei - dark blue (red when not using ErioGnost reagent)
Cartilage, mucins, mastocytes - orange to red
Background - green

Note

Microbiology staining procedures are not standardized and they depend on standard operating procedures of individual laboratories and the experience of the personnel conducting the staining procedure. Intensity of staining depends on the period of immersion in the dye. Depending on personal requests and standard laboratory operating procedures, sample processing and staining can be carried out according to other protocols.

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 use. 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. In order to avoid an erroneous result, a positive and negative control samples are advised before application.

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 which is available on demand.

Storing, stability and expiry date

Keep Safranin O kit in a tightly sealed original packaging at temperature of +15°C to +25°C. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Manufacturing and expiry date are printed on the product's label.

References

1. Carson, F. L., Hladik, C. (2009): *Histotechnology: A Self-Instructional Text*, 3rd ed., Chicago: ASCP Press
2. Kiernan, J. A. (2008): *Histological and Histochemical Methods*, 4th ed., Bloxham: Scion Publishing Ltd.

SAFO-X, V2-EN2, 12 August 2021, SK/VR

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