OIL RED O KIT

IVD In vitro diagnostic medical device

Four-reagent kit for staining fat cells and neutral fats acc. to Johnson

INSTRUCTIONS FOR USE

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

ORO-K-250 (4x250 mL)

Introduction

BioGnost's Oil Red O kit is used for histological visualization of lipids in tissues. It is used with frozen tissue sections; lipids melt if processed in xylene or in alcohol. Oil Red O dye has mostly replaced Sudan stains (Sudan III and Sudan IV) previously used for detecting lipids because it provides more intense red staining and is simpler to use.

Product description

• OIL RED O KIT- Four-reagent kit for detecting lipids

The kit contains:	100 tests (ORO-100T)	4x 250 mL (ORO-K-250)			
Oil Red O reagent	80 mL (0R0-0T-80)	250 mL (0R0-0T-250)			
Basic activation buffer	50 mL (BAP-OT-50)	250 mL (BAP-0T-250)			
Formaldehyde NB 4%	100 mL (FNB4-100)	250 mL (FNB4-250)			
Hematoxylin ML	30 mL (HEMML-0T-30)	250 mL (HEMML-0T-250)			

NOTE: Oil Red O kit is not intended for tissues embedded in paraffins because lipids in that kind of tissues melts and disintegrates.

Other slides and reagents that may be used in staining:

- · Embedding medium for cryostat sectioning in different colors, such as BioGnost's CryoFix Gel
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 models of VitroGnost glass slides
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX Low, BioMount C, BioMount Aqua, Canada Balsam
 VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- BioGnost's immersion media, such as Immersion oil. Immersion oil. types A. C. FF. 37, or Immersion oil Tropical Grade

Sample staining procedure

NOTE

Apply the reagent so it completely covers the section.

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

Pour 4% NB Formaldehyde into glass staining jars (Coplin, Hellendahl or Schifferdecker), return to original bottles after staining. Close tightly.

1.	Rinse the sample in distilled (demi) water						
2.	Prepare Oil Red O reagent working solution: mix 8 ml of Oil Red O reagent with 5 ml of Basic activation buffer. Wait for						
2.	10 min. before using the working solution						
	Note: The working solution is stable for 24 hours.						
3.	Cut to 6-10 μ m slices and mount on a VitroGnost glass slide.	2 min					
4.	Immerse and fix the section in 4% NB Formaldehyde.	1 min					
5.	Rinse under tap water						
6.	Add Oil Red O reagent working solution (\geq 5 drops)						
7.	Rinse under tap water						
8.	Stain the section using Hematoxylin ML (add \geq 5 drops)	1-3 minutes					
9.	Rinse under tap water	1-2 minutes					
10.	Apply suitable water-based covering medium (BioMount Aqua).						

b) using four-reagent 250 mL kit (ORO-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.	Rinse the sample in distilled (demi) water	
2.	Prepare Oil Red O reagent working solution depending on the amount of test sections: mix Oil Red O reagent and Basic activation buffer in 8:5 ratio, i.e. 8 volumes of Oil Red O reagent and 5 volumes of Basic activation buffer (8+5). Wait for 10 min. before using the working solution	
	Note: The working solution is stable for 24 hours.	
3.	Cut to 6-10 μ m slices and mount on a VitroGnost glass slide.	2 min
4.	Immerse and fix the section in 4% NB Formaldehyde.	1 min
5.	Rinse under tap water	3 min
6.	Immerse in Oil Red O working solution	10 min
7.	Rinse under tap water	

8.	Immerse into Hematoxylin ML	1-3 minutes
9.	Rinse under tap water	1-2 minutes
10.	Apply suitable water-based covering medium (BioMount Aqua).	

Result

Lipids - red Nuclei - blue

Note

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 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 Oil Red O kit in a tightly closed original package at temperature between 15°C and 25°C. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

- 1. Melis, M., Carpino, F., Di Tondo, U. (1989), Tecniche in anatomia patologica, Edi Ermes, Milano.
- 2. Prophet, E.B., Mills, B., Arrington, J., Sobin, L. (1968), Laboratory methods in histotechnology, McGraw Hill, Washington D.C.
- 3. Bancroft, J.D., Gamble, M. (2002), Theory and practice of Histological Techniques, Churchill Livingstone, New York.

ORO-X, V6-EN5, 24 April 2019, IŠP/VR

	Â	Refer to the supplied documentation	°c-JC°C	Storage temperature range	Σ	Number of tests in package	REF	Product code	Ce	European Conformity		BIOGNOST Ltd. Medjugorska 59 10040 Zagreb	C	E
ſ	[]i]	Refer to supplied instructions	淤	Keep away from heat and sunlight	8	Valid until	LOT	Lot number	***	Manufacturer		CROATIA www.biognost.com		
Ī	IVD	For in vitro diagnostic use only	Ť	Keep in dry place	ų	Caution - fragile					_			