BIOGNOST®

OIL RED O KIT

CE IVD *In vitro* diagnostic medical device Classified acc. to Regulation (EU) 2017/746 - **Class A** device

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

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30708STARVF	
EMDN code	W01030708	
REF **Catalog number**	**Volume**	**UDI-DI number**
ORO-100T	For 100 tests	03858890000238
ORO-K-250	4x250 mL	03858890005912

Intended use and test principle

BioGnost's Oil Red O kit is used for histological visualization of lipids in tissues. It is performed on freshly frozen tissue sections, since processing the samples in xylene or alcohol would dissolve the lipids. The staining is based on physical solubility rather than a chemical reaction. Oil Red O is soluble in lipids, but poorly soluble in water. When a sample (usually a frozen section) is exposed to dye dissolved in a solvent, the dye transfers from the solvent to lipid drops in the tissue because it dissolves easier there. 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 easier to use.

Product description

- **OIL RED O KIT** – Four-reagent kit for detecting lipids

The kit contains:	**100 tests (ORO-100T)**	**4 x 250 mL (ORO-K-250)**	**Storage temperature**
Oil red O reagent	80 mL (ORO-OT-80)	250 mL (ORO-OT-250)	15-25°C
Basic activation buffer	50 mL (BAP-OT-50)	250 mL (BAP-OT-250)	15-25°C
Formaldehyde NB 4%	100 mL (FNB4-100)	250 mL (FNB4-250)	15-25°C
Hematoxylin ML	30 mL (HEMML-OT-30)	250 mL (HEMML-OT-250)	15-25°C

Additional reagents and materials that can be used in this method

- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- Embedding medium for cryostat sectioning in different colors, such as BioGnost's CryoFix Gel

Preparation of frozen sections for staining

- Freeze the tissue as fast as possible to avoid formation of artifacts and distortion: embed the tissue sample in CryoFix Gel and freeze it.
- Mount the block on the cryostat. Optimal temperature for cryostat sectioning is -5 to -6°C for samples thicker than 15 μm. Thinner sections require a lower temperature.

NOTE

Apply the reagent so that it completely covers the section.

Sample staining procedure

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.	Prepare **Oil Red O working solution**: mix 8 ml of Oil Red O reagent with 5 ml of Basic activation buffer. Wait for 10 min. before using the working solution	
	Note: The working solution is stable for 24 hours	
2.	Section the sample into 6–10 micron-thick slices and mount them onto a VitroGnost microscope slide	2 min
3.	Immerse and fix the section in 4% NB Formaldehyde	1 min
4.	Rinse under tap water	3 min
5.	Add **Oil Red O reagent working solution** (≥5 drops)	10 min
6.	Rinse under tap water	
7.	Stain the slide using Hematoxylin ML (add ≥5 drops)	1-3 min
8.	Rinse under tap water	1-2 min
9.	Apply suitable water-based covering medium (BioMount Aqua)	

b) using four-reagent kit, 250 mL (ORO-K-250)

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.	Prepare **Oil Red O reagent working solution** depending on the amount of test samples: 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 minutes before using the working solution	
	Note: The working solution is stable for 24 hours.	
2.	Section the sample into 6–10 micron-thick slices and mount them onto a VitroGnost microscope slide	2 min
3.	Immerse and fix the section in 4% NB Formaldehyde	1 min
4.	Rinse under tap water	3 min
5.	Immerse into **Oil Red O reagent working solution**	10 min
6.	Rinse under tap water	

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

Result

Lipids – red
Nuclei – blue

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining results.

Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards. To avoid an incorrect staining result, it is advised to use a positive and negative control.
If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label and in the Safety Data Sheet, which is available on request.

Storage, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The expiration date is printed on the product 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.

Warnings and precautions regarding the materials contained in the product:



H302	Harmful if swallowed.
H312	Harmful if on skin.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H332	Harmful if inhaled.
H341	Suspected of causing genetic defects.
H350	May cause cancer.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P301+P312	IF SWALLOWED: if you feel unwell, call POISON CENTER/doctor.
P302+P352	IF ON SKIN: wash with plenty of water.
P304+P340	IF INHALED: remove person to fresh air and keep comfortable for breathing.
P305+P351+P338	IF IN EYES: rinse carefully with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.
P308+P313	IF exposed or concerned: get medical advice/attention.

ORO-IFU_ENV6, 25.02.2026., IŠP

	Manufacturer	LOT	Batch code		Consult instructions for use	Σ	Contains sufficient for <n>tests		Contains hazardous substances
	Date of manufacture	REF	Catalogue number		Caution	CE	European conformity		
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device	UDI	Unique device identifier		

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Version	Description / reason for change	Date
6	Revised acc. to Regulation (EU) 2017/746 - IVDR	25.02.2026.