

NEUTRAL RED REAGENT

IVD *In vitro* diagnostic medical device



Neutral Red aqueous counterstain solution for use with special staining kits

INSTRUCTIONS FOR USE

REF Product code: NR-OT-100 (100 mL)

Introduction

Neutral Red reagent is intended for use with various staining methods in histology and cytology. It is primarily used as nuclear stain as dye for nuclei and basophilic structures, but it can also be used as fluorochrome dye. It is used for supravital staining, as counterstain during Gram staining, for staining Nissl bodies, and as a part of Twort's histology dye for polychromatic staining. Neutral Red dye is also a pH indicator that changes color from red to yellow with 6.8 to 8.0 pH range.

Product description

- **NEUTRAL RED REAGENT** – Neutral Red aqueous counterstain solution with added stabilizers.

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 70, Histanol 80, 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

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.

Preparing cell cultures for staining

- Fix the sample (4% NB Formaldehyde, 10% NB Formaldehyde) for 10-20 minutes.
- Rinse the section under running water for 2-5 minutes twice.

Example of histology samples and cell cultures staining using Neutral Red reagent

To stain histology sections using Neutral Red reagent, pour it into a glass staining jar (Coplín, Hellendahl or Schifferdecker), return to original bottles after staining. Close tightly. Filter the reagent if necessary.

For staining cell cultures start with step number 5!

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.	Dip into (or apply) Neutral Red reagent	2-10 minutes
	Note: each cell line has its own individual optimal staining period that depends on fixing	
6.	Rinse in distilled (demi) water	2 exchanges, 2 min each
7.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
8.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 1 min each
9.	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 using VitroGnost microscope slide (Note: steps 7, 8, and 9 can be skipped while staining cell cultures).

Result

Nuclei - red

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. All the samples must be processed with the most modern technology and be visibly marked. Follow the manufacturer's instructions for handling. In order to avoid mistakes, staining must be conducted by a trained professional. Only trained medical personnel may make a diagnosis. Use only microscope according to standards of the medical diagnostic laboratory. In order to avoid an erroneous result, a positive and negative check is 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 Neutral Red reagent in a tightly closed original package at temperature between 15°C and 25°C. Keep in dry places, do not freeze and avoid exposure to direct sunlight. Expiry date is stated on the product's label.

References

1. Conn, J. (1977): *Biological Stains*, 9th ed. Baltimore: Williams and Wilkins Co.
2. Carson, F. L., Hladik, C. (2009): *Histotechnology: A Self-Instructional Text*, 3rd ed., Chicago: ASCP Press
3. Twort, F. W. (1924): An improved neutral red, light green double stain for staining animal parasites, microorganisms and tissues, *Journal of State Medicine*, 32: 351.
4. Gram, H. C. (1884): Über die isolierte Färbung der Schizomyceten in Schnitt- und Trockenpräparaten, *Fortschritte der Medizin* 2: 185-9.

NR-OT-100, V1-EN1, 29 October 2018, IŠP/VR

	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		European Conformity
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until		Lot number		Manufacturer
	For <i>in vitro</i> diagnostic use only		Keep in dry place		Caution - fragile				

 BIOGNOST Ltd.
Medjugorska 59
10040 Zagreb
CROATIA
www.biognost.com

