

# ORANGE G / ANILINE BLUE REAGENT

IVD In vitro diagnostic medical device CE

**For use with Mallory Trichrome kit**

## INSTRUCTIONS FOR USE

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

### Introduction

Orange G / Aniline Blue reagent is a component of Mallory Trichrome kit used for treating the tested microscopic sample using three different stainings with differential counterstaining of two basic parts of the tissue (muscle and collagen fibers) in focus. Sample staining starts with Fuchsin Acid acidic dye that causes nuclei and muscles get stained red to pink. Phosphotungstic acid molecule then shuts out Fuchsin Acid dye molecules from collagen and thus enables Aniline Blue to bind, resulting in collagen being stained contrast blue in relation to previously used red dye. Orange G (molecule of the lowest molar mass) stains erythrocytes.

### Product description

- **ORANGE G / ANILINE BLUE REAGENT** - Reagent containing Orange G and Aniline Blue dye.

### Usage of Orange G / Aniline Blue reagent as a component of Mallory Trichrome kit

#### Other sections and reagents necessary for 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.
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX Low Eco, BioMount C, BioMount Aqua, Canada Balsam
- 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
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade
- Other components of Mallory Trichrome kit: Fuchsin Acid reagent (product code FA-OT-100) and Phosphotungstic acid, 1% solution (product code FMK1-OT-100)

### 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  $\mu\text{m}$  slices and place them on a VitroGnost glass slide.

### Sample staining procedure

Pour the reagents into glass staining jars (Coplín, 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 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Immerse into Fuchsin Acid reagent	30 seconds
6.	Rinse in distilled (demi) water	until the excessive dye is washed off of the section
7.	Immerse into Phosphomolybdic acid, 1% solution	3 min
8.	Remove excessive reagent from the section using filter paper (without rinsing in distilled water)	
9.	Immerse in Orange G/Aniline Blue reagent	4-6 minutes
10.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
11.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
12.	Dehydrate using 100% alcohol (Histanol 100)	2 min
13.	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

Muscle fibers, cytoplasm, nuclei - red to pink  
 Collagen - blue  
 Erythrocytes - orange to red

## 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 Orange G / Aniline Blue reagent in a tightly sealed original packaging at temperature of +15 to +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.

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	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				



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