BIOGNOST®

# AZOPHLOXINE powder dye, C.I. 18050

IVD *In vitro* diagnostic medical device CE

## Red 2G, Acid Red 1

## INSTRUCTIONS FOR USE

REF Catalogue number: AZO-P-5 (5 g) AZO-P-25 (25 g)

**Introduction**

Histology, cytology and other related scientific disciplines study the microscopic anatomy of tissues and cells. In order to achieve a good tissue and cellular structure, the samples need to be stained in a correct manner. Azophloxine powder dye is used as an alternative to Biebrich Scarlet dye in Masson and Goldner trichrome staining methods.

**Product description**

- **AZOPHLOXINE powder dye** - Powder dye for making solution for histology staining

**Example of use of Azophloxine staining solution in Masson Goldner trichrome kit**

**Other reagents and chemicals not used in the method:**

- Glacial acetic acid
- Ponceau 2R powder dye
- Fuchsin Acid powder dye
- Hematoxylin, Weigert A, Ferri reagent, Weigert B, P.T.A.-P.M.A. reagent, Fast Green F.C.F. reagent, 1% solution of acetic acid (components of BioGnost's Masson Goldner trichrome kit, product codes MGT-100T, MGT-K-100, MGT-K-500)

**Preparing the solutions for staining**

Ponceau 2R/Fuchsin Acid solution

- Mix 1.5 g of Ponceau 2R and 0.5 g of Fuchsin Acid poweder dyes and dissolve them in 98 ml of distilled (demi) water
- Add 2 ml of glacial acetic acid

Azophloxine solution

- Dissolve 0.5 g of Azophloxine powder dye in 99.4 mL of distilled (demi) water.
- Add 0.6 ml of glacial acetic acid

Ponceau 2R/Fuchsin Acid/Azophloxine working solution

- Mix 12 ml of Ponceau 2R/Fuchsin Acid solution with 8 ml of Azophloxine solution.
- Add 80 ml of 0.2% of acetic acid solution (made by mixing 0.16 ml of glacial acetic acid and 79.84 ml of distilled (demi) water)

**Preparing the histological sections for staining**

- Fix the tissue sample tightly (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 fit the sample in paraffin (BioWax Plus 56/58, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6 $\mu$m slices and place them on a VitroGnost glass slide.

**Sample staining procedure**

| | | |
|---|---|---|
| **1.** | Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New) | 3 exchanges, 10 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 | 3 min |
| **5.** | Stain using Hematoxylin, Weigert A (5 drops) and Ferri reagent, Weigert B (5 drops) | 5 min |
| **6.** | Rinse under tap water | 3 min |
| **7.** | Rinse in distilled (demi) water | 3 min |
| **8.** | Stain using Ponceau 2R/Fuchsin Acid/Azophloxine working solution | 5 min |
| **9.** | Treat with 1% acetic acid solution | 15 seconds |
| **10.** | Treat with PTA-PMA reagent | 20 min |
| **11.** | Treat using 1% acetic acid solution | 15 seconds |
| **12.** | Stain using Fast Green F.C.F. reagent | 5 min |
| **13.** | Treat using 1% acetic acid solution | 3 min |
| **14.** | Dehydrate using 70% alcohol (Histanol 70) | 5 dips |
| **15.** | Dehydrate using 95% alcohol (Histanol 95) | 5 dips |
| **16.** | Dehydrate using 100% alcohol (Histanol 100) | 2 min |
| **17.** | Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New) | 2 exchanges, 5 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 a VitroGnost cover glass.

**Result**

Nuclei - black
Muscle fibers, keratin, cytoplasm - bright red
Collagen, mucus - green
Erythrocytes - red-orange

**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 Azophloxine powder dye in a tightly sealed original packaging 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.
4. Yuehuei H.A. et Martin, K.L. (2003): Handbook of Histology Methods for Bone and Cartilage, 1st edition, Springer Science+Buisness Media New York



AZO-X, V1-EN1, 24 March 2016, IŠP/VR


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

CE