Papanicolaou Orange G6

Cytoplasmic staining reagent acc. to Papanicolaou Counterstain for monochromatic staining of samples in cytology

MenidiMedica
Biotechnology Applications

REF 70770-1000: 1 lt,
70770-5000: 5 lt.

24 months, storage at RT

Introduction

OG-6 reagent is an alcoholic solution of Orange G dye with added phosphotungstic acid (PTA). The first step in using the Papanicolaou staining method implies nuclear staining with a hematoxylin solution, and next two steps consist of contrast staining using the monochromatic OG-6 reagent and one of the polychromatic EA reagent formulations consisting of two acid dyes, the Eosin Y and Light Green SF. The Orange G molecule stains the cytoplasm, and in later stages of the procedure it remains only in the mature, keratinized cells that turn different shades of orange. The third step consists of using of one of the polychromatic EA solutions that stains the unstained cellular components, such as squamous cells, nucleoli, cilia, and erythrocytes. Test samples can be gynecological and non-gynecological, such as sputum, urine, and cytological puncture samples. In order to obtain optimal staining results, the properties of OG-6 reagent are completely in accordance with other MenidiMedica Biotech reagents used for cytological staining acc. to Papanicolaou - OG-6, EA 31 reagent, as well as alternative counterstain polychromatic stains, such as EA 50 reagent and EA 65 reagent.

Product description

Counterstain for monochromatic staining of samples in exfoliative cytology. Contains BSC-certified Orange G dye with added phosphotungstic acid and required stabilizers.

Preparing the cytological smear for staining

There are two methods of collecting and preparing the cytological samples:
1. After collecting the cytological sample, place it on the microscope slide, fixate it immediately with a fixative in a spray bottle (Biofix), dry it and keep until the staining process. Cytological sample may be fixated and kept until staining by immersing into 95% alcohol solution for a minimum of 30 minutes.
2. Using liquid-based cytology method (LBC - KaryoPrep) and brush for collecting cytological samples, fixate the sample immediately (KaryoPrep Solution for fixation) by removing the brush head and immersing it in the fixative. At the beginning of processing the sample, isolate the cells from the fixative (one of the methods is to centrifuge the fixative) and place them on the microscope slide equally in a single layer. Cytological sample prepared in such a way is ready for staining. Ask MenidiMedica Biotech for the KaryoPrep LBC Application Sheet.

The Papanicolaou staining method, PROGRESSIVE

The first stage of staining procedure depends on the method the cytological sample was collected and fixated on the microscope slide. If the sample is dry and previously fixed using Biofix, it is necessary to keep it in a 95% alcohol solution for 10 minutes in order to remove polyglycols. If the section was fixated with a 95% alcohol solution, ignore this step. During staining cytology samples (prepared by using the KaryoPrep liquid based cytology method (LBC)) rehydration by descending series of alcohol solutions is not necessary. The procedure starts by rinsing the section using distilled water and is then stained using Hematoxylin.

- Rehydrate in descending series of alcohols 95, 70 and in distilled water - 10 dips in each of the 3 exchanges
- Staining using Hematoxylin - 30 seconds

Note: Longer exposure of the section to Hematoxylin reagent may also stain cyoplasm (apart from nucleus)

- Rinse the section with tap or distilled water - 30 seconds
- Blue using Scott's solution or Bluing reagent - 1 min

Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water - 3-5 minutes

- Dehydrate in ascending series of alcohols 70, 95 - 10 dips in each of the 2 exchanges
- Stain using OG-6 reagent - 2 min
- Rinse using 95% alcohol in two exchanges - 30 seconds during each of the 2 exchanges
- Stain with EA 50 reagent - 4 min
- Rinse using 95% alcohol in two exchanges - 1 minutes in each of the 2 exchanges
- Dehydrate in 100% alcohol in two exchanges (Histanol 100) - 1 minutes in each of the 2 exchanges
- Clear the section in xylene or in xylene substitute in two exchanges - 2 minutes in each of the 2 exchanges

Immediately after clearing apply an appropriate mount medium for covering/mounting on the section. If xylene was used, use one mounting xylene-based media. If xylene substitute was used, use the appropriate covering agent. Cover the section with cover glass.

Papanicolaou staining method, REGRESSIVE

The regressive staining method creates a better sample differentiation and clearer nuclear structure visibility. The first stage of staining procedure depends on the method the cytological sample was collected and fixated on the microscope slide. If the sample is dry and previously fixed using Biofix, it is necessary to keep it in a 95% alcohol solution for 10 minutes in order to remove polyglycols. If the section was fixated with a 95% alcohol solution, ignore this step. During staining cytology samples (prepared by using the KaryoPrep liquid based cytology method (LBC)) rehydration by descending series of alcohol solutions is not necessary. The procedure starts by rinsing the section using distilled water and is then stained using Hematoxylin reagent.

- Rehydrate in descending series of alcohols 95, 70) and in distilled water - 10 dips in each of the 3 exchanges
- Staining using Hematoxylin reagent - 4 min
- Rinse the section with tap or distilled water - 30 seconds
- Differentiation using 0.1% Acid alcohol - 5-10 seconds

Note: This step removes excessive hematoxylin from the nucleus and cytoplasm. Discoloration of the nuclei can occur if the section is treated with the differentiation agent for too long.

- Rinse the section with tap or distilled water - 10 dips
- Blue using Scott's solution or Bluing reagent - 1 min

Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water - 3-5 minutes

- Dehydrate in ascending series of alcohols 70 and 95 - 10 dips in each of the 2 exchanges
- Stain using OG-6 reagent - 2 min
- Rinse using 95% alcohol in two exchanges - 30 seconds during each of the 2 exchanges
- Stain with EA 50 reagent - 4 min
- Rinse using 95% alcohol in two exchanges - 1 minutes in each of the 2 exchanges
- Dehydrate in 100% alcohol in two exchanges (Histanol 100) - 1 minutes in each of the 2 exchanges
- Clear the section in xylene or in xylene substitute in two exchanges - 2 minutes in each of the 2 exchanges

Immediately after clearing apply an appropriate mount medium for covering/mounting on the section. If xylene was used, use one of mounting xylene-based media. If xylene substitute was used, use the appropriate covering agent. Cover the section with cover glass.

Note

In the case of subsidence in the Hematoxylin solution or formation of metallic glow on the surface, reagent should be filtered before use. Time periods of staining procedures are not completely standardized. The suggested methods are in accordance with MenidiMedica Biotech reagents' properties and correspond to longtime clinical and laboratory practice. Intensity of staining depends on the period of exposure to stains and reagents. Staining procedure can be changed according to personal preferences if they correspond to the basic principles of cytotechnology.

Results

Nuclei - blue -
Keratinized cells - yellow-orange
Superficial squamous epithelial cell, erythrocytes, nucleoli, cilia - pink-red
Cytoplasm of all the other cell types (parabasal and intermediate squamous cells, columnar cells, polymorphonuclear leukocytes, lymphocytes, histiocytes, adenocarcinomas, undifferentiated carcinoma cells) - green

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 MenidiMedica Biotech material safety data sheet.

Storing, stability and expiry date

Keep OG-6 reagent 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. Papanicolaou, G.N. (1941): Some improved methods for staining vaginal smears. J Lab Clin Med.
2. Papanicolaou, G.N. (1942): A new procedure for staining vaginal smears. Science.
3. Carson, F.L., Hladik C. (2009): Histotechnology: A self-instructional text, 3rd ed. ASCP Press.
4. Sherwani, R.K., Khaqn, T. et al. (2007): Conventional Pap Smear and Liquid Based Cytology for Cervical Cancer Screening – A Comparative Study, Journal of Cytology, 24 (4): pp 167-172.

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