



2E-306

Papanicolaou solution 2B

In-vitro diagnostic agent

Description

The product 2E-306 is a ready-to-use solution for professional users in histology and cytology. The product is supplied in 3 different pack sizes: 2E-306.00100 (100ml bottle), 2E- 306.00500 (500ml bottle) and 2E-306.02500 (2.5l canister)

Main components

Methanol (CAS 67-56-1)	95%
Orange II (C.I.15510)	4.7 g
Phosphotungstic acid (CAS 12501-23-4)	0.2 g

Purpose

"Papanicolaou's solution 2B" is used for cell diagnostics for the examination of histological samples (e.g. histological sections). It is a ready-to-use solution that, together with other in vitro diagnostics from our portfolio, makes cytological targets in gynaecological and clinical cytological samples evaluable.

Sample material and sample preparation

Sampling may only be carried out by qualified personnel. All samples must be processed with state-of-the-art technology. All samples must be clearly labelled.

Sample material: Sections of formalin-fixed, paraffin-embedded tissue (3–4 µm thick paraffin sections) or frozen sections as well as gynaecological and non-gynaecological samples such as sputum, urine, effusions, irrigation fluids, smears from fine needle aspiration biopsies.

Test principle

In the first step of staining, the cell nuclei are stained with a haematoxylin solution either progressively or regressively.

When staining with haematoxylin, a distinction is made between progressive staining, in which staining is carried out to the end point and then blued and preserved in tap water, and the regressive method. In the regressive method, the colour is overstained with haematoxylin and the excess colour is removed again in acidic differentiation steps and blued in tap water. With regressive staining, the nuclear structures appear more differentiated and are better visible.

In the second step, the cytoplasm is stained with an orange staining solution, which distinctly displays the mature and keratinised cells. The target structures are stained orange in different intensities.

In the third staining step, a polychrome solution, which is a mixture of eosin, light green SF and bismarck brown, is used. The polychrome solution is used to show the differentiation of the squamous epithelium.

With Papanicolaou's solution 2B, a stronger, reddish staining pattern is achieved in mature and keratinised squamous epithelia.



Staining



If necessary, deparaffinise the tissue sample and rinse it before staining. It is recommended to filter the solution before use and not to dilute it.

Example of a **progressive** staining protocol:

The staining should be done in a staining cuvette. The slides must be immersed in the solutions and moved briefly; simply placing them in the solutions gives insufficient staining results. For an optimal dyeing result, the indicated times should be observed.

Prior to staining, the sections must be deparaffinised and immersed over a descending ethanol series (96%-80%-70%- 50%, 10 seconds each) in distilled water (20 seconds). The staining is done with Papanicolaou's solution 1A Harris for 3 minutes. The samples are then rinsed under running water for 3 minutes and transferred to Papanicolaou's solution 2A orange G via an ascending ethanol series (70%-80%-96%, 30 seconds each). Staining in Orange G solution is done for 3 minutes. Then the samples are immersed 2x in ethanol 96% for 30 seconds each. Then stain with Papanicolaou's solution 3A EA 31 for 3 minutes. The samples are left 2x in ethanol 96% for 30 seconds and then incubated in ethanol 100% for 5 minutes. Then the samples are incubated in a mixture of ethanol and xylene (1+1) for 2 minutes. Finally, the samples are incubated for 2x 5 minutes in xylene and covered for the subsequent microscopy.

Example of a **regressive** staining protocol:

The staining should be done in a staining cuvette. The slides must be immersed in the solutions and moved briefly; simply placing them in the solutions gives insufficient staining results. For an optimal dyeing result, the indicated times should be observed.

Prior to staining, the sections must be deparaffinised and immersed over a descending ethanol series (96%-80%-70%- 50%, 10 seconds each in distilled water (10 seconds). The staining is done with Papanicolaou's solution 1A Harris for 6 minutes. The samples are then rinsed in distilled water for 10 seconds and then immersed in 0.1% hydrochloric acid for 10 seconds and rinsed again in distilled water. Then incubate the samples in 1.5% sodium hydrogen carbonate solution for 1 minute and rinse under running tap water for 3 minutes. The samples are transferred to Papanicolaou's solution 2B via an ascending ethanol series (70%-80%-96%, 30 seconds each). Staining in Orange G solution is done for 3 minutes. Then the samples are immersed 2x in ethanol 96% for 30 seconds each. Then stain with Papanicolaou's solution 3A EA 31 for 3 minutes. The samples are left 2x in ethanol 96% for 30 seconds and then incubated in ethanol 100% for 5 minutes. Then the samples are incubated in a mixture of ethanol and xylene (1+1) for 2 minutes. Finally, the samples are incubated for 2x 5 minutes in xylene and covered for the subsequent microscopy.

Result

Cytoplasm (with additional staining with Papanicolaou's solution 3A EA 31)

cyanophilic (basophilic)	blue-green to green
eosinophil (acidophil)	pink
keratinised	pink-orange
Nuclei	blue to dark purple
Erythrocytes	red
Microorganisms	grey-blue, grey-green



Precautionary measures

When removing the product, care must be taken to avoid contamination of the storage vessel. Once the solution has been removed, it must not be returned to the canister. If turbidity occurs or there are solids, the product must be discarded. The product is intended for single use and must not be reused.

Storage and shelf life

Store the unopened containers in a dry place at 15 to 25 °C, avoiding direct sunlight. The shelf life is 2 years. See also the best-before date (BBD) on the label. Once the containers have been opened, the shelf life corresponds to the best-before date, as long as the storage conditions are observed and the solution is handled properly.

Safety notice

If any serious incidents occur in connection with the product, please report them to the manufacturer and the national authority.

Literature

Romeis, Mikroskopische Technik, Editors: Maria Mulisch, Ulrich Welsch, 2010, Springer Spektrum, 18th edition