Technical Data Sheet

Giemsa stain (Azur-Eosin-Methylene blue)

for biochemistry

Order number: 3107

Giemsa stain and the corresponding staining method named after Gustav Giemsa was originally developed for the detection of plasmodia, but quickly found other applications as the Giemsa staining mixture brought properties that were of great value for histology: The chromatin and nuclear membrane stain well with Giemsa and the staining of the cytoplasm varies according to cell type (Giemsa shows metachromasia).
The Giemsa dye (powder) is composed of Azure B, Methylene blue and Eosin. Giemsa stain is a nucleic acid stain attaching DNA regions with high amounts of adenine-thymine bonding (Karyotyping - or more specifically "G-banding" -is based on this specificity) - but not alone. While the basic dye Methylene blue reliably stains acidic cellular components (especially the nucleus), the acidic dyes Azure B and Eosin show an affinity for the cytoplasm, granules and other basic cellular components.
The staining results are analyzed under the microscope.

Application

The Giemsa stain is the standard diagnostic procedure for malaria (histopathological diagnosis) and other blood parasites and is further used in hematology and cytogenetics.
Giemsa stain is used for staining of peripheral blood smears, specimens obtained from the bone marrow and other cytological materials. It is further used for G-banding (Giemsa-banding) in cytogenetics to stain the chromosomes and identify chromosomal aberrations.
Another method for which Giemsa is used is white blood cell differential counts (often combined with Wright or May-Grunewald stain).

Preparation of a stock solution

The standard stock solution contains

- 3.8g of Giemsa powder
- 250 ml of Methanol (e.g. LC-7011)
- 250 ml of Glycerol (anhydrous, e.g. LC-5996)

About how to proceed, there are different possibilities (Giemsa dissolved in methanol first, Giemsa dissolved in Glycerol first, heating of one or the other preparation or the whole mixture to 60°C…).

Independently of which protocol is used, it is recommended to finally filter the solution to remove remaining solid particles. Store in a dark glass bottle in a cool place and avoid direct sunlight. The solution needs to stand a period of time (at least 1 week) prior to use.

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Caution: Do not contaminate the Giemsa stock solution with water; even the smallest amount of water will worsen the staining and make it increasingly ineffective.
Avoid shaking the Giemsa stock solution for at least 24 hours before use to avoid re-suspending of precipitates that may form.

Preparation of a working solution

The final dye concentration of the stain and of course the exact procedure vary depending on the application (e.g. for thin blood smears a 1:20 dilution of the Giemsa stock solution is used, while for thick blood smears a 1:50 dilution is common). The working solution is usually prepared in phosphate buffer pH 7.2.

Results: Microscopic observations of different cells and parasites

The table below shows typical staining results, i.e. the colors that can be expected for a Giemsa staining of the specific cell types and organisms.
However, the staining results clearly depend on the exact composition of the Giemsa solution and are influenced by the pH value, the buffer substances, staining time and type of fixation.

Object of investigation	Color observed
Bacteria	Pale to dark blue
Basophils	Purple nuclei, blue coarse granules
Borrelia spirochetes	Mauve-purple
Chlamydia trachomatis (inclusion bodies)	Blue-mauve to dark purple depending on the stage of development
Eosinophils	Purple nuclei, faintly pink cytoplasm, red to orange granules
Erythrocytes (red blood cells)	Mauve-pink
Leucocytes (white blood cells)	Dark purple to magenta nuclei, pale blue or grey-blue cytoplasm
Lymphocytes	Dark blue nuclei, light blue cytoplasm
Melanin granules	Dark green to black
Monocytes	Purple nuclei, pink cytoplasm
Neutrophils	Red-purple nuclei, pink cytoplasm
Platelets	Violet-purple granules
Plasmodium (Malaria parasites)	Red-pink nucleus, blue cytoplasm
Yersinia pestis	Blue with dark stained ends (bipolar staining)

JB14012022