May-Gruenwald - Solution Microscopy grade

Eosin-Methylene blue - Solution acc. to May-Grünwald
Product No. A0415

Description

The **May-Gruenwald solution** contains two dyes, Eosin and Methylene blue. AppliChem May-Gruenwald solution (A0415) is suitable as a ready-to-use solution for simple differential staining of fixed blood or bone marrow. Furthermore, the solution is used in dilution for use in panoptic staining according to Pappenheim. In the Pappenheim procedure for staining of blood elements diluted May-Gruenwald solution is used in combination with Giemsa solution (AppliChem product no. A0885).

Note: The staining effect is pH dependent. Therefore use buffer solutions throughout for dilutions and rinsing. For this purpose we recommended the usage of Sörensen phosphate buffer 0.1 M (pH 6.8-7.0): mixing 4.0 mL of stock solution A (0.1 M KH_2PO_4) and 6.0 mL of stock solution B (Na_2HPO_4 x $2H_2O$, 0,1 M) gives a final pH value of approx. 7. Store buffer solutions at 2-8°C.

Storage: at ambient temperature, protect from light

Literature: Romeis, Mikroskopische Technik, 18. Aufl., Spektrum Akadem. Verlag (German)

Protocol 1

May-Gruenwald staining for cytological preparations (smears)
from: Romeis, A3.103

Solutions:

1. May-Gruenwald - solution for microscopy (A0415)
2. Phosphate buffer according to Sörensen (please see above)

Method:

For the staining of air-dried smears

1. stain in May-Gruenwald solution, 3-5 min
2. to each slide add 1 mL of phosphate buffer acc. to Sörensen (0.1 M, pH 7), 5-10 min
3. drain staining solution from slides
4. rinse thoroughly with phosphate buffer acc. to Sörensen (0.1 M, pH 7), 2x 1 min
5. dry slides on air and mount

Result:
Nuclei: blue-purple (reddish)
Cytoplasm: reddish-orange, gray / blue / blue

Result in the staining of blood smears:
Nuclei of leukocytes / early stage of erythrocytes: blue-purple (reddish)
Cytoplasm of erythrocytes: red
Cytoplasm of lymphocytes: blue
Cytoplasm of monocytes: pigeon-blue
Eosinophilic granules: brick-red
Basophilic granules: blue-purple (reddish)
Thrombocytes: purple

protocol 2

Staining according to May-Gruenwald-Giemsa (Pappenheim stain)
for cytological preparations (smears)
from: Romeis, A3.105

Solutions:

1. Phosphate buffer according to Sörensen
2. May-Gruenwald working solution: Dilute May-Gruenwald-solution for microscopy (A0415) 1:2 using phosphate buffer acc. to Sörensen, filter the solution
3. Giemsa working solution: dilute Giemsa solution for microscopy (AppliChem Product No. A0885) 1:10 using phosphate buffer acc. to Sörensen, filter the solution

Method:

For the staining of air-dried smears.

1. place slides in methanol, 5-10 min
2. stain in May-Gruenwald working solution, 5-8 min
3. drain staining solution from the slide

   (no rinsing step here)
4. stain in Giemsa working solution, 10 min
5. rinse thoroughly with phosphate buffer acc. to Sörensen, 2 x 1 min
6. dry slides and mount

Result:
Nuclei: reddish-purple (blue)
Cytoplasm: reddish / orange / gray / light blue / light blue

Result in the staining of blood smears:
Nuclei of leukocytes / early stage of erythrocytes: reddish-purple (blue)
Cytoplasm of erythrocytes: pink
Cytoplasm of lymphocytes: light blue
Cytoplasm of monocytes: gray-blue
Eosinophilic granules: brick red to orange
Basophilic granules: dark purple
Neutrophil granules: violet light

Protocol 3

Panoptic staining according to Pappenheim
for slices
from: Romeis, instructions A3.72

For the staining of tissue sections fixed according to Maximow or Helly and embedded in paraffin

Solutions:

1. Neutral (pH 7) distilled water.

   Prepared e.g. by mixing phosphate buffer acc. to Sörensen (pH 7.0) with distilled water at a ratio of 1:10
2. May-Gruenwald working solution: mix freshly before use May-Gruenwald solution for microscopy (A0415) with distilled water in the ratio 1:8
3. Giemsa working solution: dilute freshly before use Giemsa solution for microscopy (AppliChem Product No. A0885) with distilled water in the ratio 1:75
4. Various solvents for histology

Method:

1. deparaffin sections in descending ethanol series, and finally distilled water
2. rinse in neutral distilled water
3. pre-stain in May-Gruenwald working solution, 20 min at 35° C in an incubator
4. completely drain the dye solution (do not rinse) and stain sections with Giemsa working solution, 40 min at 35°C in an incubator
5. (You may differentiate briefly using 0.15% acetic acid)
6. rinse in neutral distilled water
7. pressing with filter paper
8. dehydrate in acetone-ethanol (1:1)
9. brighten in xylene and mount

Result:
Staining of blood cells on slices is similar to that on smears. Connective tissue is stained more intensely than by using Giemsa alone

Nuclei: reddish-purple
Eosinophilic granules: brownish-orange to brick-red
Basophilic granules: ultramarine (possibly slightly purple)
Neutrophil granules: bluish-pink to brownish
Cytoplasm of lymphocytes: light blue
Cytoplasm of monocytes: light blue, possibly with fine red granules
Erythrocytes: pink

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