Product Information May-Grünwald Giemsa

Intended Use

May-Grünwald's eosin methylene blue and Giemsa's azure eosin methylene blue are intended to be used for staining of blood and bone marrow smears and cytological specimens, such as urine sediment or sputum. For staining of most histology specimen (mostly gastric sections) Giemsa is used.

Principle

The purple colour of cell nuclei, is due to molecular interaction between eosin Y and an azure B-DNA complex. The intensity of the staining depends on the azure B content and on the ratio azure B/eosin Y. The pH of the solutions, fixation and staining time can influence the staining result.

Composition

May-Grünwald is alcohol based and contains May-Grünwald's eosin methylene blue and methanol (>85%). Giemsa is alcohol based and contains Giemsa's azure eosin methylene blue, methanol (>50%) and glycerol.

Stability and Storage

May-Grünwald and Giemsa are stable for five years. The bottles must be kept closed. The advised storage temperature is 18 - 30°C. Used solutions and solutions that are past their shelf-life must be disposed of, according to local disposal guidelines.

Procedures for bone marrow, cytology samples and blood smears

A. General method for Bone marrow or Cytology specimen:

1. Prepare Sørensen buffer solution pH 7:
   Dilute 100 ml of the concentrated Sørensen phosphate buffer with 1.9L de-ionized water.
2. Prepare the May-Grünwald working solution:
   Dilute 250 ml May-Grünwald solution with 250 ml Sørensen buffer solution pH 7.
3. Prepare the Giemsa working solution:
   Dilute 50 ml Giemsa solution with 450 ml Sørensen buffer solution pH 7.
4. Proceed according to the table below

Reagent sequence	Minutes
Undiluted May-Grünwald	3
May-Grünwald working solution	5
Move slides gently in Sørensen buffer pH 7	1
Move slides gently in Giemsa working solution	25
Flush in tap or de-ionized water	

B. General method for whole blood smears:

1. Prepare Sørensen buffer solution pH 7:
   Dilute 100 ml of the concentrated Sørensen phosphate buffer with 1.9L deionized water.
2. Prepare the May-Grünwald working solution:
   Dilute 250 ml May-Grünwald solution with 250 ml Sørensen buffer solution pH 7.
3. Prepare the Giemsa working solution:
   Dilute 50 ml Giemsa solution with 450 ml Sørensen buffer solution pH 7.
4. Proceed according to the table below

Reagent sequence	Minutes
Methanol or undiluted May-Grünwald	3
May-Grünwald working solution	5
Move slides gently in Sørensen buffer pH 7	1
Move slides gently in Giemsa working solution	20
Flush in tap or de-ionized water	

Note: When using methanol in the first step as fixative, basophilic granules might be missed. This is known from literature.

C. Traditional method according to Pappenheim for whole blood smears

1. Prepare Sørensen buffer solution pH 7:
   Dilute 100 ml of the concentrated Sørensen phosphate buffer with 1.9L de-ionized water.
2. Prepare the Giemsa working solution:
   Dilute 25 ml Giemsa solution with 475 ml Sørensen buffer solution pH 7.
3. Proceed according to the table below

Reagent sequence	Minutes
Undiluted May-Grünwald	3
Flush in de-ionised water	1
Move slides gently in Giemsa working solution	20
Flush in tap or de-ionised water	

D. Quick staining method for whole blood smears

1. Prepare Sørensen buffer solution pH 7:
   Dilute 100 ml of the concentrated Sørensen phosphate buffer with 1.9L de-ionized water.
2. Prepare the Giemsa working solution:
   Dilute Giemsa solution 1 to 6 up to 1 to 8 with Sørensen buffer solution pH 7.
3. Proceed according to the table below

Reagent sequence	Minutes
Undiluted May-Grünwald	2-3
Move slides gently in Sørensen buffer pH 7	1
Move slides gently in Giemsa working solution	4-5
Flush in tap or de-ionised water	

E. Staining method for whole blood smears using a bridge

1. Prepare Sørensen buffer solution pH 7:
   Dilute 100 ml of the concentrated Sørensen phosphate buffer with 1.9L de-ionized water.
2. Prepare the Giemsa working solution:
   Dilute Giemsa solution 1 to 1 up to 1 to 4 with Sørensen buffer solution pH 7.

3. Proceed according to the table below

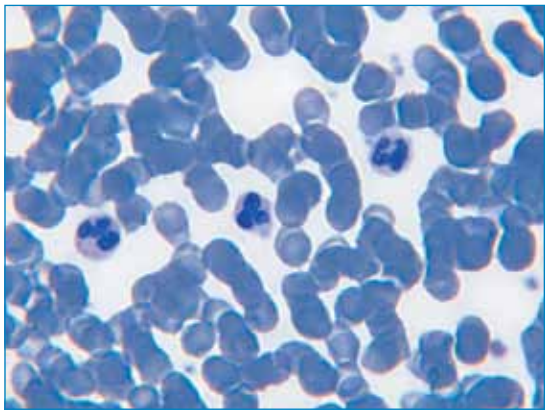
Reagent sequence	Minutes
Undiluted May-Grünwald	2-3
Flush in tap or de-ionized water	1
Giemsa working solution	4-5
Flush in tap or de-ionized water	

Note: The times as listed in the tables are approximates and can be adjusted to suit personal preferences. Staining solutions will lose their staining power when heavily used and the staining times should be longer or fresh solutions should be used.

Performance Characteristics

Type Blood-cell	Amount	Characteristics
RBC	4 – 6 x 10^{12}/liter	Pink/brown discs; clearer in the middle due to their concave structure
PLT	0.2 – 0.3 x 10^{12}/liter	Purple coloured granules; much smaller then RBC
NEUT	50 – 70 % *	Transparent, pink/blue cytoplasm; 2-5 lobed bright purple nucleus
EO	2 – 4 %	Typical pink-orange granulated cytoplasm; generally 2-lobed purple nucleus
LYM	20 – 40 %	Transparent purple cytoplasm; one large, purple-pink nucleus
MONO	3 – 8 %	Largest of the leukocytes; transparent, pink/blue cytoplasm with horseshoe-shaped pink/purple nucleus
BASO	0.5 – 1.0 %	Granulo-rich cytoplasm exhibiting dark-blue stain overruling the dark-blue nucleus stain

* From total WBC population; generally 5 – 7 x 10 cells/liter



• May-Grünwald stained blood cells (100x)

Troubleshooting Bone marrow, Cytology samples and Blood smears

Insufficient colour development of leucocytes

- Prepared smears not completely dried
- Glass slides used are not degreased or pre-treated
- Exhausted May-Grünwald or Giemsa working solution

Too much erythrocyte fragments

- Specimen older than 12 hours
- Blood smear dried at ambient temperature > 30°C
- Prepared smear not completely dried

Colour too much blue and red

- Too long staining in May-Grünwald and Giemsa
- Too short colour development in Sørensen buffer or de-ionized water
- Ambient temperature > 30°C

Colour intensity Bone marrow, Cytology samples and Blood smears

Varying times may influence the colour intensity of both the blue and red:

Step May-Grünwald	Step Giemsa	Blue	Red
+ 5 min.	+ 5 min.	++	++
+ 5 min.	same time		+
same time	+ 5 min.	+	
– 5 min.	– 5 min.	––	––
– 5 min.	same time		–
same time	– 5 min.	–	

Procedure for Histology samples

1. Prepare the Giemsa working solution:
   Add 20 ml Giemsa solution to 80 ml de-ionized water.
   It is important to add the Giemsa to the water and not vice versa.
   Filter the solution before use.
2. Prepare the differentiation solution:
   Add 4 drops of Glacial Acetic Acid (96%) to 100 ml of de-ionized water.
   Measure the pH of the solution. It should be 3.0 – 3.2.
3. Proceed according to the tables below

♦ De-paraffination of tissue

Reagent sequence	Minutes
UltraClear/Xylene	3x1
Ethanol 100%	3x1
Ethanol 70%	1
Flush with tap water	1

♦ Staining of tissue

Reagent sequence	Minutes
Insert 3 times in de-ionized water	
Giemsa working solution (use only once)	30
Differentiation fluid (differentiation to purple)	dip just once
Ethanol 96% (differentiation to blue)	dip just once
Isopropanol (2-propanol)	dip just once
Isopropanol (2-propanol)	3 x 2
UltraClear/Xylene (refresh each time)	3 x 2
Coverslip slides	

Performance Characteristics for Histology samples

Nucleus	: blue / violet
Cytoplasm	: blue
Erythrocytes	: pink
Eosinophilic granules	: orange
Basophilic granules	: purple

Troubleshooting for Histology samples

Insufficient colour development
- Glass slides used are not degreased or pre-treated
- Exhausted Giemsa working solution

Colour too much blue
- Too long staining in Giemsa
- Ambient temperature > 30°C
- Slide too short in differentiation fluid

Colour too much red instead of purple
- Too much glacial Acetic Acid in differentiation fluid
- Slide to long inserted in differentiation fluid

Poor colour development of blue
- Too long inserted in Ethanol 96%

Colour intensity Histology samples

Varying staining times may influence the colour intensity of both the blue and red:

Step May-Grünwald	Step Giemsa	Blue	Red
+ 5 min.	+ 5 min.	++	++
+ 5 min.	same time		+
same time	+ 5 min.	+	
– 5 min.	– 5 min.	––	––
– 5 min.	same time		–
same time	– 5 min.	–	

Frequently Asked Questions

Do I have to change my working protocol?

When changing to J.T.Baker there's no real need to change the whole procedure. For an optimized performance we refer to the Product Information.
See scheme above.

Are the solutions ready to use?

This depends on the application. We refer to the methods advised. In general May-Grünwald is used undiluted or diluted 1 to 1. Giemsa is always diluted in the range from 1:5 up to 1:20.

For which specimen types is May-Grüwald and Giemsa suitable?

Blood smears, bone marrow and tissue material, such as gastric sections.

Features and Benefits

Optimized formulation	Colours are balanced
Optimized working procedure available	Colour tones easy adaptable
Sørensen buffer solution 3716 available	Easy diluting (if needed), reproducible results
Traditional production method	Avoids crystallization
Suitable for hematology, histology and cytology	Broadly applicable

Ordering Information

Product	Product Number	Packaging
May-Grünwald	3855.0500	500 ml
May-Grünwald	3855.1000	1 liter
May-Grünwald	3855.2500	2.5 liter
Giemsa	3856.0500	500 ml
Giemsa	3856.1000	1 liter
Giemsa	3856.2500	2.5 liter
Sørensen buffer concentrate	3716	10 x 100 ml

Phillipsburg, NJ 9001:2008 & 14001:2004
Paris, KY 9001:2008
Mexico City, Mexico 9001:2008
Deventer, the Netherlands 9001:2008, 14001:2004 & 13485:2003
Gliwice, Poland 9001:2008 & 17025:2005
Selangor, Malaysia 9001:2008
Dehradun, India 9001:2008, 14001:2004 & 13485:2003
Mumbai, India 9001:2008 & 17025:2005

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Ordering Information and Assistance

Customer Service
TEL: +31-570-687500
FAX: +31-570-687574
E-MAIL: avantor.emea@avantormaterials.com
www.avantormaterials.com

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Corporate Headquarters

Avantor Performance Materials, Inc.
3477 Corporate Parkway
Suite #200
Center Valley, PA 18034
USA

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