Technical Data Sheet

Technovit® 7100 Routine Staining, Enzyme Histochemistry According to Gerrits

#14653

HEMATOXYLIN-EOSIN

Staining process

1. Stain the sections in hematoxylin in accordance with Gill* (filtrate the dye solution)	15 min
2. Blue in tap water	10 min
3. Rinse in aqua dest.	
4. Counterstain sections with Eosin	2-5 min
5. Dehydrate through ethanol 96% and 100%	
6. Clarify with xylen and cover in Eukitt	

Result

Nucleus	blue
Basophilic cytoplasm	blue
Acidophilic cytoplasm	pink
Muscle tissue	pink
Connective tissue	pink

Solutions

Hematoxylin in accordance with Gill	
Hematoxylin (C.I. 75290)	6g
Sodium iodate	0,6g
Aluminum sulphate	52,8g
Aqua dest.	690 ml
Ethylene glycol	250 ml
Glacial acetic acid	60 ml

Eosin	
Eosin Y-(alcoholic) C.I. 45380	0,5g
Ethanol 96%	100 ml
Glacial acetic acid	2 drops

* After staining with hematoxylin (1) the plastic matric can be decolorized with 0.5 ml of HCL (36%) in ethanol 70%: briefly submerse and then quickly process in tap water (2).

PERIOD ACID SCHIFT (PAS)

Staining process

1. 0,4% periodic acid	30 min, 56°C
2. Rinse in tap water	
3. Aqua dest. rinse 3x	
4. Schiff's reagent	15 min
5. Rinse thoroughly in tap water	
6. Rinse in aqua dest.	
7. Counterstain sections with hematoxylin in accordance with Gill	10 min
8. Blue in tap water	10 min
9. Dehydrate, clarify with xylen and cover in Eukitt	

Note: To avoid a specific pink sheen, one can rinse with sulphite water instead of tap water (5), see also Feulgen.

Result

Nucleus	blue
Glycogen	violet / red
Basement membranes	violet / red
Mucin	violet / red

Solutions

Schiff's reagent

Solution 1: Pararosaniline (C.I. 42500) 1 N hydrochloric acid	 0,5g 15 ml
Solution 2: Potassium metabisulphite ($K_2S_20_5$) Aqua dest.	 0,5g 85 ml

Mix solution 2, solution 1. After 24 hours (in the dark) the light brown solution is decolorized with 200 mg of bone black (approx. 2 min) and subsequently filtrated.

Store the colorless reagent (leucofuchsin) in the refrigerator.

Gill's hematoxylin: see Hematoxylin-Eosin

FEULGEN

Staining process

1. Hydrolise in hydrochloric acid 5 N	20 min ZT
2. Rinse in aqua dest. 3 x	
3. Schiff's reagent	15 min
4. Sodium hydrogen sulphite 0.5%	3 x 2 min
5. Rinse thoroughly with tap water	
6. Dehydrate, clarify with xylen and cover in Eukitt	

Result

DNS	violet / red
Other tissue elements	colorless

Solutions

Schiff's reagent

Hydrochloric acid 5 N

Fill up with 42 ml of hydrochloric acid 36% up to 100 ml Sodium.

$NaHSO_3$	0,5g
Aqua dest.	100 ml

GIEMSA

Staining process

1. Stain sections in the Giemsa solution (20%) (Giemsa Merck: Dilute 1:5 with aqua dest.)	1,5 hrs ZT
2. Briefly in acetic acid solution: 4 drops to 100 ml of aqua dest.	2 sec
3. Submerse in alcohol 96%	
4. Submerse in alcohol 96%	
5. Isopropanol	3 x 2 min
6. Clarify with xylen and cover in Malinol	

Result

Nucleus	violet
Cytoplasm	blue
Erythrocytes pink	pink

PRUSSIAN BLUE REACTION IN ACCORDANCE WITH PERLS

Staining process

1. Potassium ferrocyanide First warm up the solution to 60°C and then filter filtrates	15 min
2. Rinse in aqua dest.	
3. Safranin O. 0,2%	2-5 min
4. Rinse in acetic acid 1%	
5. Dehydrate, clarify with xylen and cover in Eukitt	

Result

Nucleus	red
Hemosiderin	blue / green

Solutions

<u>Potassium ferrocyanide solution</u>	
Potassium ferrocyanide	1g
Aqua dest.	50 ml

Hydrochloric acid 2%	50 ml
Safranin-Solution	
Safranin O. (C.I. 50240)	0,2g
Acetic acid 1%	100 ml

PERIODIC ACID METHENAMINE SILVERC (PAMS) ACCORDING TO JONES

Note: It is recommended to stick on the plastic sections with Mayer's albumin.

Staining process

1. Periodic acid 1%	30 min
2. Rinse in aqua dest. 3x	
3. Methenamine silver solution	60 min, 60°C
4. Rinse in aqua dest., microscopic test. Sections that have been too weakly stained again in 3	
5. If the sections refuse to dissolve despite pre-treatment, dry them on a plate at 60°C in accordance with Point 4.	
6. Gold chloride 0,2%	1-2 min
7. Rinse in aqua dest.	
8. Sodium thiosulphate 2%	5 min
9. Rinse in tap water	
10. If necessary, counterstain with HE or safranine O	
11. Dehydrate, clarify with xylen and cover in Eukitt	

Result

Basement membranes	brown / black

Solutions

Methenamine silver tock solution	
a) Hexamethylenetetramine 3 %	100 ml
b) Silver nitrate 5%	5 ml

a) and b) can be stored separately	
Methenamine silver stain solution	
Stock solution	50 ml
Borax 5%	5 ml
Periodic acid:	1% (Sigma No. P 7875)
Gold chloride:	0,2%
Sodium thiosulphate solution:	2% ($Na_2S_2O_3.5H_20$)

DETERMINING ENZYME ACTIVITY

Determination of the enzyme activity in tissues that are embedded in 2 hydroxyethyl methacrylate (GMA) - in particular Technovit® 7100.

Freshly removed tissue is fixated in 4% neutral formaldehyde at 4°C for two hours (immersion). If perfusion fixations are made, very brief fixation times can be adhered to and the enzyme activity is better maintained.

Rinsing fluid

0.1 M cacodylate buffer pH 7.4; the material can possibly be left overnight at 4°C.

Dehydration

1. Alcohol 70% acetone 70%, 30 min at 4°C
2. Alcohol 96% acetone 96%, 30 min at 4°C
3. Alcohol 100% acetone 100%, 30 min at 4°C

Pre-infiltration

4. Alcohol 100% Technovit® 7100 1:1, 2 hrs at 4°C
or
Acetone 100% Technovit® 7100 1:1, 2 hrs at 4°C

Infiltration

Technovit® 7100, 12 hrs at 4°C

Polymerization

15 parts Technovit® 7100 (solution A)
1 part Technovit® 7100 hardener II, at 4°C

The tissue can be embedded in Histoforms S or Q, or in the Sorvall embedding system. Because polymerization starts at 4°C, it will occur slower than at room temperature. A polymerization time of 12 hours at 4°C must be adhered to ensure polymerization.

The 2-µ sections are also dried at room temperature on aqua dest. Enzyme actions can be made without removing the plastic matrix.

Note: It is difficult to detect dehydrogenases.

ALKALINE PHOSPHATASE IN ACCORDANCE WITH BURSTONE

Staining process

1. Incubate the plastic sections in the incubation medium. Note: In many cases a 2-hour incubation period is sufficient.	1-3 hrs
2. Rinse in aqua dest.	2 min
3. Counterstain the sections with nuclear fast red	5-10 min
4. Rinse in aqua dest.	
5. Air dry	
6. Cover in malinol	

Result

Nucleus	red
Enzyme activity area	blue

Note: In this reaction the choice of medium used to cover the material is significant because crystals formation may occur in the reaction product.

Solutions

<u>Buffer solution</u>	
0.2 M tris-(hydroxymethyl)-aminomethane	2,4g
Aqua dest.	100 ml
Set the pH value to 8.9 with diluted HCL and store the buffer at 4°C.	
<u>Incubation medium</u>	

Naphtol AS-MX phosphate, disodium salt (Sigma)	5g
N,N dimethylformamide	0,25 ml
After dissolving, add:	
Aqua dest.	25 ml
Buffer solution (pH 8,9)	25 ml
$MgSO_4.7H_2O$ 10%	2 drops
Fast Blue BB (Sigma)	30 mg

Shake well and then filtrate before using.

Note: Always freshly prepare the incubation medium.

ATP-ASE (WACHSTEIN AND MEISEL)

Staining process

1. Incubate the plastic sections in the incubation medium (filtrate before using) Note: In many cases a 2-hour incubation period is sufficient.	1-3 hrs, 37°C
2. Rinse in aqua dest.	2 min
3. Sodium sulphide solution	30 sec
4. Rinse in aqua dest.	
5. Counterstain the sections with nuclear fast red	5-10 min
6. Rinse in aqua dest.	
7. Air dry	
8. Cover with Eukitt or malinol	

Result

Nucleus	red
Enzyme activity area	brown

Solutions

1. Tris maleic acid buffer pH 7.2 solution A Maleic acid	29g

Tris-(hydroxymethyl)-aminomethane	30,3g
Aqua dest.	500 ml
Add 2g of activated carbon, shake for ten minutes and filtrate. Then add 40 ml of the stock solution A, 20 ml 1N NaOH, and fill with aqua dest. up to 100 ml (pH 7.2).	
2. Lead nitrate solution	
Lead nitrate	2g
Aqua dest.	100 ml
3. Magnesium sulphate solution	
$MgSO_4.7H_2O$	1,2g
Aqua dest.	100 ml
Incubation medium	
Aqua dest.	22 ml
Disodium adenosine-5-triphosphate (Boehringer, Mannheim)	25 mg
Tris maleic acid buffer pH 7,2	20 ml
Magnesium sulphate solution	5 ml
Lead nitrate solution (add by drops), heat to 42°C and filtrate	3 ml
Sulphide solution	
Sodium sulphide	2g
Aqua dest.	100 ml
Adjust the pH value to 7.0-7.5 with 1 N of HCL (verify with pH paper).	

Source of Information

Heraeus Kulzer, 2014