Technical Data Sheet

Technovit® 9100 Routine staining, Immune Reactions, Enzyme Histochemistry, In-Situ Hybridization

EMS Catalog #14655

The following staining and detection reactions are only important examples of processing hard-cut sections. They also apply to MMA thin sections.

Reagents, antibodies, probes, detection seystems are variable.

1. Routine staining

Counterstaining the sections for immunohistochemistry and enzyme histochemistry	
Hematoxylin n. Mayer flowing water in tap water transfer to aqua dest.	30 sec./RT 10 min/RT
Rinse nuclear fast red in aqua dest.	10 min/RT
Methyl green (cleaned; see Romeis) Rinse with aqua dest.	10-20 min/RT
HE staining	
Same as staining paraffin sections	
Giemsa staining	
Deacrylate sections	
Giemsa sol. (Mix fresh!)	30-40 min/RT
Differentiate and dehydrate	
Acetone / xylol (95 : 5)	
Acetone / xylol (70 : 30)	
Acetone / xylol (30 : 70)	
Xylol	
Masson Goldner staining	
Deacrylate sections	

Haemalaun (Mayer)	10 min/RT
Tap water	
Ponceau acid magenta azophloxin	45 min/RT
1% Acetic acid	
Phosphomolybdic acid / Orange G	7 min/RT
1% Acetic acid	
Light green	40 min/RT
1% Acetic acid	
Ascending alcohol series	
Xylol	
Cover with Eukitt or similar	

2. Complete Immune Reaction

Antibody Incubation	
Rinse in 0.01M phosphate buffer (pH 7.4)	
Primary antibody Diluted in DAKO Antibody Dilutent	16 h/4°C or 30-45 min/RT
Rinsing buffer	
DDAKO EnVision Polymer (GAM/GAR), AP paired	
Detection reaction	
Rinsing buffer	
Substrate chromogen solution: Fast Red	15-20 min/RT
Counterstaining with hematoxylin to Mayer	

3. Execution of enzyme histochemistry

Alkaline and acid phosphatase	
Rinse in 0-1M tris buffer (pH 9.4)	10 min/RT

Incubation in the reaction solution 0.1M tris buffer (pH 9.4) Real blue salt Naphthol-AS-BI-phosphate	2 h/37°C
Rinse in aqua dest.	
Rinse in 0-1M acetate buffer (pH 5.6)	10 min/RT
Incubation in the reaction solution 0.1M acetate buffer (pH 5.6) Hexanium-pararosaniline solution Naphthol-AS-BI-phosphate	1 h/37°C
Rinse in aqua dest.	
Refixate in 4% formalin	2-3 h/RT
Rinse in tap water	
Counterstain with methyl green	
Naphthol-AS-D chloroacetate esterase (ASD)	
Reinse in 0.01M phosphate buffer (pH 7,4)	5 min/RT
Incubation in the reaction solution 0.01M phosphate buffer (pH 6.5) Naphthol-AS-D-Chloroacetate Hexanium-pararosaniline solution	1 h/RT
Rinse in aqua dest.	
Counterstain with hematoxylin to Mayer	

Source of Information

Heraeus Kulzer, 2014