Instructions for use

Hematoxylin solutions A and B acc. to Weigert

Art. No.: X906; X907

To get the working solution (*iron hematoxylin solution*) mix both solutions A and B at a ratio of 1:1 prior to use. The working solution is stable for about 8 days at room temperature. It is used when an acidoresistant staining of nuclei is required, e.g. when working with delimed tissues or for counterstaining during trichrome staining. Pure hematoxylin stainings will fade under acid conditions after a time and, therefore, are not suitable.

Masson Goldner Trichrome Staining

A successive staining method with three different staining solutions allowing a differentiated visualisation of various fibers of connective tissue against epithelial and muscle tissue.

Goldner's stain I: Muscle tissue and cytoplasm red (ponceau), connective tissue also red (acid fuchsine)
Goldner's stain II: Erythrocytes orange (orange G), connective tissue without colour (phosphotungstic acid)
Goldner's stain III: Connective tissue green (light green SF)

Principle of staining:
The dyes differ in particle size: *Goldner's stain I* contains a fine-particle phase (ponceau), which infiltrates quickly all structures of tissue, and a coarse-particle phase (acid fuchsine), which works more slowly. At first it stains only the coarse structures of tissue by masking the fine-particle phase. You have to stop the staining procedure at that point to avoid an overstaining of the tissue.
During the differentiation step with *Goldner's stain II* (phosphotungstic acid) it is important to decolour the connective tissue as much as possible. Only then it can be stained with *Goldner's stain III (*light green).

Additional chemicals required:

- Goldner's stain I (Ponceau-Fuchsine Art. No. 3469)
- Goldner's stain II (Phosphotungstic acid - Orange G, Art. No. 3470)
- Goldner's stain III (Light green SF yellowish, Art. No.3473)
- Acetic acid solution 1% (Acetic acid 100%, Art. No.3738)
- Usual CLEARing Agents: ROTI®Histol (Art. No. 6640)
  ROTICLEAR® (Art. No. A538)
  Xylene p.a. (Art. No. 4436)
- Appropriate Mounting Media: ROTI®Histokitt (Art. No. 6638), compatible with ROTI®Histol
  ROTI®Mount (Art. No. HP68), compatible with ROTICLEAR®
  ROTI®Histokitt II (Art. No. T160), compatible with Xylene

Instruction*: *For de-waxed, rehydrated specimens.*

1. Stain with iron hematoxylin solution acc. to Weigert (Mix solution A+B at a ratio of 1:1) max. 3 min	6. Rinse with acetic acid solution 1% 30 sec
2. Blue in flowing tap water 10-15 min	7. Counterstain with Goldner's stain III 2-5 min *Optionally: Examine by microscope.*
3. Stain with Goldner's stain I 5-10 min	8. Wash with acetic acid solution 1% 2-5 min
4. Rinse with acetic acid solution 1% 30 sec	9. Dehydrate by ascending alcohol series 10. CLEAR with ROTI®Histol, ROTICLEAR® or xylene 11. Mount with appropriate mounting medium
5. Stain with Goldner's stain II until decolouration of connective tissue **1-3 min (normally sufficient) up to 30 min** *Examine by microscope. Specimens may not dry out.*	**Please note:** Instead of light green solution it is possible to use a 0.1-0.2% solution of anilinblue (Art. No. 4002) for counterstaining (step 7).

* Acc. to Romeis, Mikroskopische Technik, 18. Auflage, Spektrum Akademischer Verlag (2010)

Result: Cell nuclei: dark brown; Cytoplasm, muscle: red; Erythrocytes: orange; Connective tissue: green

Van Gieson Trichrome Staining

This staining method allows a differential visualisation of tissue structures in paraffin sections, especially the collagen connective tissue.

Van Gieson's solution contains two dyes with very different properties: The fine-particle picric acid infiltrates quickly all structures of tissue by staining them yellow. The coarse-particle acid fuchsine can stain only the coarse structures of collagen connective tissue during the short residence time. There the picric acid is masked. Do not prolong the residence time to avoid the masking of picric acid in other tissue structures, too (principle of progressive staining).
After staining remove the picric acid as completely as possible from collagen connective tissue for tissue stained with acid fuchsine tends to fade out when being exposed to acids and bases.
The procedure demands some skill for you have to stop rinsing before the picric acid is also removed from the other tissue structures (in that case the tissue becomes become reddish).

Additional chemicals required:

- Van Gieson's solution (Art.. No. 3925)
- Ethanol denatured: 99.8 % (Art. No. K928), 96 % (T171), 70 % (T913)
- HCl-ethanol solution 3 % (Art. No. 6477) – working solution 0.5 %
- Usual CLEARing Agents: ROTI®Histol (Art. No. 6640)
  ROTICLEAR® (Art. No. A538)
  Xylene p.a. (Art. No. 4436)
- Appropriate Mounting Media: ROTI®Histokitt (Art. No. 6638), compatible with ROTI®Histol
  ROTI®Mount (Art. No. HP68), compatible with ROTICLEAR®
  ROTI®Histokitt II (Art. No. T160), compatible with Xylene

Instruction*:

1. De-wax and rehydrate sections (descending alcohol series finishing off with ethanol 70 %).	7. Stain with van Gieson's solution. 1-3-min
2. Stain with iron hematoxylin solution acc. to Weigert (Mix solution A + B at a ratio of 1:1, solution stable for 8 days at room temp.). 5-10 min	8. Rinse shortly with ethanol 70% and ethanol 96%. *Caution, picric acid is especially soluble in diluted ethanol!*
3. Rinse with distilled water to avoid precipitation of hematein.	9. Dehydrate and rinse with ethanol 96%, finish with 2 x ethanol 100%.
4. *Examine by microscope: Nuclei should be grey blue, cytoplasm colorless to max. light grey.* If the cytoplasm is stained too intensive differentiate in HCl-ethanol 0.5%. 2-3 sec	10. CLEAR with CLEARing agent.
	11. Mount with appropriate mounting medium.
5. Rinse in tap water to interrupt the differentiation.	**Please note at step 9:** *Rinse moderately with highly concentrated ethanol to remove the picric acid from the connective tissue (see also above).*
6. Blue in flowing tap water. 10 min	

* Acc. to Romeis, Mikroskopische Technik, 18. Auflage, Spektrum Akademischer Verlag (2010)

Result:

- Cell nuclei: dark blue / dark brown
- Collagene fibres: red
- Muscle, cytoplasm: yellow

Elastica van Gieson Staining

Van Gieson trichrome staining is well combinable with elastica staining acc. to Weigert allowing a good overview of various tissue structures, especially a differentiated visualisation of connective tissue and elastic fibres.

Additional chemicals required:

- Van Gieson's solution (Art. No. 3925)
- Ethanol denatured 99.8 % (Art. No. K928), 96 % (T171), 70 % (T913)
- Resorcinol fuchsine solution acc. to Weigert (Art. No. X877)
- Usual CLEARing Agents: ROTI®Histol (Art. No. 6640)
  ROTICLEAR® (Art. No. A538)
  Xylene p.a. (Art. No. 4436)
- Appropriate Mounting Media: ROTI®Histokitt (Art. No. 6638), compatible with ROTI®Histol
  ROTI®Mount (Art. No. HP68), compatible with ROTICLEAR®
  ROTI®Histokitt II (Art. No. T160), compatible with Xylene

Instruction*:

1. De-wax and rehydrate sections (descending alcohol series finishing off with ethanol 80 %).	9. Rinse with distilled water to avoid precipitation of hematein.
2. Stain with resorcinol fuchsine solution. 20-30 min	10. Blue in flowing tap water. 10 min
3. Rinse with tap water until stain fades.	11. Stain with van Gieson's solution. 1-3-min
4. Rinse with distilled water.	12. Rinse shortly with ethanol 70% and ethanol 96%. *Caution, picric acid is especially soluble in diluted ethanol!*
5. Differentiate with ethanol 80%.	13. Dehydrate and rinse with ethanol 96%, finish with 2 x ethanol 100%.
6. Rinse with distilled water to interrupt the differentiation.	14. CLEAR with CLEARing agent.
7. Examine by microscope: Elastic fibres dark violet, background light rose.	15. Mount with appropriate mounting medium.
8. Stain with iron hematoxylin solution acc. to Weigert (Mix solution A + B at a ratio of 1:1, solution stable for 8 days at room temp.). 2-3 min	**Please note at step 13:** *Rinse moderately with highly concentrated ethanol to remove the picric acid from the connective tissue and, therefore, avoid fading of the staining. Caution: If the rinsing is too intensive the tissue becomes reddish!*

*Acc. to Romeis, Mikroskopische Technik, 18. Auflage, Spektrum Akademischer Verlag (2010)

Result:

- Elastic fibres: dark violet
- Cell nuclei: dark blue / dark brown
- Collagene fibres: red
- Muscle, cytoplasm: yellow

Please note:
The colour intensity depends on the pre-treatment and the composition of the samples to be stained.
It may initially be necessary to adapt the method to the respective conditions.

Danger H225-H319-H336

Hematoxylin solution A acc. to Weigert
X906.1 500ml

Danger H290-H318

Hematoxylin solution B acc. to Weigert
X907.1 500ml

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