Instructions for use

Resorcinol Fuchsine Solution according to Weigert

X877

Resorcinol fuchsine solution acc. to Weigert is generally used for reproducing elastic fibres in microscopy, e.g. in elastica staining acc. to Weigert.

Elastic fibres are typical connective tissue fibres, which can be highly compressed and stretched. They are composed of the proteins elastin and fibrillin and build very compact tissue structures with only little interspaces. Elastic fibres are primarily found in elastic vascular walls.

Counterstaining of nuclei is normally carried out with nuclear fast red aluminium sulphate solution. If acid-proof staining is necessary - e.g. for elastica van Gieson staining - the nuclei are stained with iron hematoxylin solution acc. to Weigert (see below).

Elastica Staining according to WEIGERT

Additional chemicals required:

- Nuclear fast red aluminium sulphate solution (Art. No. N069)
- Ethanol denatured: 99.8% (Art. No. K928), 96% (T171), 70% (T913)

- 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%).	6. Rinse with distilled water.
2. Stain with resorcinol fuchsine solution acc. to Weigert. 10-30 min	7. Dehydrate by ascending alcohol series finishing with 2 x ethanol 100%.
3. Rinse with tap water until stain fades.	8. Clear with clearing agent.
4. Stain with nuclear fast red aluminium sulphate solution. 5-10 min	9. Mount with appropriate mounting medium.
5. Rinse with tap water.	

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

Result:

- Elastic fibres: dark violet
- Cell nuclei: red
- Cytoplasm: light red

Elastica Staining according to HART

Additional chemicals required:

- Nuclear fast red aluminium sulphate solution (Art. No. N069)
- Ethanol denatured: 99.8% (Art. No. K928), 96% (T171), 70% (T913)
- HCl-ethanol 3% (Art. No. 6477) – working solution 1 %

- 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%).	5. Rinse with distilled water.
2. Stain with a solution made up of 5 ml resorcinol fuchsine solution acc. to Weigert and 100 ml HCl-ethanol 1%. 10-24 h	6. Dehydrate by ascending alcohol series.
3. Rinse with tap water. 15 min	7. Clear with clearing agent.
4. Stain with nuclear fast red aluminium sulphate solution. 10 min	8. Mount with appropriate mounting medium.

*Acc. to Burck, Histologische Technik, 6. Auflage, Thieme (1988)

Result:

- Elastic fibres: deep brown
- Cell nuclei: red

Elastica van Gieson Staining

Elastica staining can be well combined with other staining methods. A subsequent staining of connective tissue with van Gieson's solution (picrofuchsin) allows a good overview of different tissue structures.

- Staining of elastic fibres with *resorcinol fuchsine solution acc. to Weigert.*

- Staining of nuclei with *iron hematoxylin solution acc. to Weigert.*
  With this solution the nuclei are stained in an acid-proof way making them resistant against picric acid, an ingredient of van Gieson's solution.

- Staining of connective tissue with *van Gieson's solution*.
  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 too long. 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 reddish).

Additional chemicals required:

- Iron hematoxylin solution acc. to Weigert
  (Solution A: Art. No. X906, solution B: Art. No. X907)
- Ethanol denatured: 99.8% (Art. No. K928), 96% (T171), 70% (T913)
- Van Gieson's solution (Art. No. 3925)

- 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 shortly 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 shortly 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-H290-H302+H312+H332-H318-H336-H370

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Resorcinol fuchsine solution acc. to WEIGERT	
X877.1	**500ml**
X877.2	**1l**
X877.3	**2,5l**