Trichrome Stain Kit

Catalog No.: K053

Size:7*50mL(100T)

Storage: The kit should be stored at room temperature for one year, avoid light.

Kit components:

Item	Size	Storage
Reagent A1	25 mL	RT，avoid light
Reagent A2	25 mL	RT，avoid light
Mix equal parts of A1 and A2 to form Iron Hematoxylin solution, which is stable for about 4h.		
Reagent B：Acid alcohol differentiation solution	50 mL	RT
Reagent C：bluing solution	50 mL	RT
Reagent D：Ponceau-acid fuchsin solution	50 mL	RT，avoid light
Reagent E：Acetic acid solution	50 mL	RT
Reagent F：Phosphomolybic acid solution	50 mL	RT，avoid light
Reagent G：Aniline blue solution	50 mL	RT，avoid light

Introduction

Trichrome stain kit is intended for use in the study of connective tissue, muscle and collagen fibers. It is mainly used in distinguishing collagen from smooth muscle since these two component look similar under the microscope.

This kit may be used on formalin-fixed, paraffin-embedded or frozen sections.

Assay Procedure(for reference only)

1. Dewax to distilled water.
2. Stain with Iron hematoxylin solution for 5-10mins.
3. Differentiate with Acid alcohol differentiation solution for 10-15s.
4. Blue in Bluing solution for 2-5mins. Rinse in deionized water.
5. Stain with Ponceau-acid fuchsin solution for 5-10mins. Rinse in deionized water.
6. Differentiate with Phosphomolybic acid solution for 1-2mins or till collagen is not red.
7. Without rinsing, add Aniline blue solution to section and stain for 1-2mins.
8. Place section in Acetic acid working solution(Mix 1 part of Acetic acid solution and 2 part deionized water） for 1min.
9. Dehydrate very quickly in 95% ethanol 2-3s, then 5-10s for three times in absolute ethanol and

transparent in xylene.

10. Seal with resinene.

Result

Nucleus	Black
Cytoplasm, Muscle fibers	Red
Collagen	Blue

Note

1. Slice dewaxing should be as clean as possible.
2. Equal amount of A1 and A2 were mixed to form Iron Hematoxylin solution, which generally lost its dyeing ability within 24h.
3. Tissue fixation plays very important role. Different fixatives can prolong or shorten teh dyeing time.
4. In the classic trichromatic staining, Garris hematoxylin was used to stain the nucleus, but the color of the section after Garris hematoxylin staining was not bright enough. Weigert hematoxylin was used to stain the nucleus in this staining solution, because the purpose of staining is mainly to distinguish collagen fibers and muscle fibers, and this staining step can also be omitted generally.
5. The differentiation time of acid ethanol should be determined according to the thickness of slice, the type of tissue and the old or new.
6. Weak acid solution can make the color more clear and bright. If it is used in a large amount, it can be replaced by 0.1%-0.3% acetic acid solution. If aniline blue is over dyed, step9 can be omitted and aniline blue can be dyed directly after molybdophosphoric acid cleaning.
7. The differentioation of phosphomolybdic acid should be controlled under microscope until the collagen fibers are light red and the fibers are red. The differentioation time depends on the depth of staining , generally 1-2mins.