

Masson's Trichrome Stain

Product Number: S100045

Shipping and Storage

Store at room temperature, with a shelf life of 12 months.

Component

Component	S100045	S100046
Solution A	20mL	100mL
Solution B	20mL	100mL
Solution C	20mL	100mL
Solution D	20mL	100mL
Solution E	20mL	100mL
Solution F	20mL	100mL

Description

Masson's trichrome staining, also known as Masson staining, is a classic method for collagen fiber staining. This technique utilizes the differential permeability of anionic biological dyes with varying molecular weights in connective tissues. Smaller molecular weight dyes easily penetrate densely structured, poorly permeable tissues, while larger molecular weight dyes can only enter loosely structured, highly permeable tissues, resulting in distinct coloration of different tissue components.

This product, the Masson Trichrome Staining Solution, is a kit with the following main components: Solution A is a 2.5% potassium dichromate mordant, Solution B and Solution C are mixed in equal volumes to form Weigert's iron hematoxylin stain, Solution D is alizarin red acid fuchsin, Solution E is a 1% phosphotungstic acid solution, Solution F is a 2.5% toluidine blue solution. After staining connective tissue sections with the Masson Trichrome Staining Solution, collagen fibers appear sky blue to bright deep blue, while muscle fibers, cytoplasm, cellulose, keratin white, and other components appear red to purplish red, and red blood cells appear light red.

Protocol

1. Slice preparation: Paraffin sections are deparaffinized to water; -Frozen sections stored at 20°C need to be allowed to stand and restored to room temperature.
2. Soak the slices in solution A and incubate in a 65°C oven for 30 minutes. Rinse with tap water for 30 seconds until the yellow color on the tissue fades away. Preheat both liquid D and liquid F in a 65°C oven simultaneously.
3. Mix equal volumes of solution B and solution C (ready to use, not pre prepared for storage), slice and immerse in the mixture for 1 minute, then rinse with running water.
4. Slices were differentiated with 1% hydrochloric acid alcohol (concentrated hydrochloric acid: anhydrous ethanol=1:100) for about 1 minute until the cell nucleus turned grayish black and the background was almost colorless or light gray.
5. Gently wash with tap water, drain excess water from the slices, immerse the slices in solution D for 6 minutes, and the tissue will appear bright red. If the red color is too light, the staining time can be appropriately extended. If it is a frozen section (8μm), the staining time is shortened to 3 minutes. Then rinse the slices with running water for about 20 seconds until the water flowing down the slices becomes colorless.
6. Slice and drain the water slightly (do not dry the slices), soak in solution E for about 1 minute. This step is for differentiation, until the collagen fibers appear light red and the fibers are red. The time of the E solution can be adjusted according to the depth of staining, usually 1-2 minutes.
7. After slightly draining the E solution from the slices, they are directly stained with F solution without washing. For routine tissues, it usually takes 2-30 seconds; for bone tissues, it takes 30 seconds, and for skin tissues, it takes 60 seconds. If it is a

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frozen slice (8μm), the staining time needs to be appropriately shortened and adjusted according to the degree of staining.

8. Slices were washed and differentiated in three consecutive tanks of 1% glacial acetic acid aqueous solution for about 7 seconds each, with the aim of differentiating excess aniline blue. When rinsing with a 1% glacial acetic acid aqueous solution in the third tank, conduct a microscopic examination to avoid excessive differentiation of the blue color.
9. Slices were dehydrated in three consecutive cylinders of anhydrous ethanol for approximately 3 seconds, 5 seconds, and 5 seconds. Transparent with xylene for 5 minutes and sealed with neutral gum.

Note

1. The temperature is lower in winter, so it is recommended to heat up solution A, solution D, and solution F before use, and the corresponding dyeing time also needs to be adjusted.
2. After treatment with solution A, the time for washing the slices with water should not be too long, as it may result in a lighter and darker overall staining effect.
3. E-liquid can be reused, but if the dye solution changes color significantly, it needs to be replaced with a new one.
4. Each set of staining solution (20mL specification) can be used for staining approximately 80 slices. When the staining of tissues or cells is significantly lighter or abnormal, please replace with a new staining solution.
5. Please wear lab clothes and disposable gloves during operation.