

MASSON'S TRICHOME KIT

Intended Use

Masson's Trichrome is used to differentiate between collagen and smooth muscle in fixed tissue. Three dyes are employed to selectively stain muscle, collagen fibers, fibrin, and erythrocytes.

Principle and Results

Masson's Trichrome staining is based on the principle of differential staining, where different tissue components absorb specific dyes according to their chemical properties. This technique utilizes three primary dyes: Weigert's hematoxylin, which stains cell nuclei; Beirich Scarlet-Acid Fuchsin, which targets the cytoplasm, muscle, and collagen; and Aniline Blue, which highlights collagen fibers. The selective affinity of these dyes for various tissue elements allows for a clear distinction between muscle, collagen, and other connective tissues.

Specimen Criteria

Deparaffinized and re-hydrated 4-5 μ tissue sections on positively charged slides.

Precautions

Masson's Trichrome kit should be used by properly trained individuals. Refer to SDS for complete warning, precautions, hazard, and precautionary statements, and disposal information.

Autostainer Configuration

This stain kit may be adapted for use on most open platform autostainers when purchased in larger sizes.

Quality Control

Ethos Biosciences tests each lot using appropriate tissue controls: normal liver. Follow all recommended Quality Control procedures detailed with your laboratory.

Interpretation

Collagen and mucin	Blue
Muscle fibers, cytoplasm, keratin	Red
Nuclei	Blue/Black

Prestaining Procedures:

1. Preheat Bouin Solution to 56 – 62°C in oven or water bath.
2. Prepare fresh Weigert's Working solution by combining Weigert's Hematoxylin (A) and Weigert's Iron Chloride (B) with a 1 to 1 ratio. Use a chemically cleaned container or new/unused plasticware. Mix thoroughly.

PROCEDURE						
#	ACTION	SOLUTION	HEAT	TIME		DETAILS
				MIN	SEC	
1	DEPARAFFINIZE	XYLENE, 2 CHANGES	–	5	–	5 MINUTES EACH CHANGE
2	RINSE	GRADED ALCOHOL	–	1	–	11 MINUTE EACH CHANGE
3	RINSE	RUNNING TAP WATER	–	1	–	
4	INCUBATE	BOUINS	56-62°C	60	–	COVER SOLUTION WITH LID OR EQUIVALENT
5	WASH	TAP WATER	–	5	–	DI WATER RINSE
6	IMMERSE	WEIGERT'S WORKING SOLUTION	–	10	–	
7	WASH	TAP WATER	–	5	–	DI WATER RINSE
8	IMMERSE	BIEBRICH SCARLET-ACID FUCHSIN	–	5	–	RINSE WITH 3 CHANGES OF DI WATER
9	IMMERSE	PHOSPHOMOLYBDIC/ PHOSPHOTUNGSTIC ACID	–	10-15	–	DO NOT RINSE
10	IMMERSE	ANILINE BLUE	–	10-15	–	RINSE WITH 3 CHANGES OF DI WATER
11	IMMERSE	1% ACETIC ACID	–	1	–	RINSE WITH 3 CHANGES OF DI WATER
12	DEHYDRATE	GRADED ALCOHOLS	–	1	–	1 MINUTE EACH CHANGE
13	CLEAR	XYLENE, 2 CHANGES	–	5	–	5 MINUTES EACH CHANGE
14	COVERSLIP	RESINOUS MEDIA	–	–	–	

Limitations

1. Results will largely depend on proper and adequate specimen collection and fixation.
2. Carryover of solution from one jar to the next may result in cloudiness, lack of contrast, and water artifacts. Change solutions regularly.
3. If using a xylene substitute, follow manufacturer's recommendation for deparaffinization and clearing steps.

Notes

1. Use a "same slide control". This refers to a tissue section stained with a known positive control on the same slide for the stain being used. This allows pathologists to directly compare the stain.
2. Bouins step is critical for proper staining. Acidic pH is necessary to increase the selectivity of aniline blue.
3. Bouins can be performed at room temperature, slides must be incubated overnight if the solution is not heated.
4. Phosphomolybdic/phosphotungstic acid acts as a mordant, do NOT rinse when transferring to aniline blue.
5. Fading of the aniline blue is commonly caused by over differentiation of the slide in 1% acetic acid.

References

1. Sheehan, Dezna C. and Barbra B. Hrapchak. *Theory and Practice of Histology*. 2nd ed. St. Louis: Mosby, 1980. 191-192.
2. Carson, Freida L. and Christa Hladik. *Histotechnology: Self-instructional Text*. 3rd ed. Chicago, III.: American Society of Clinical Pathologist, 2009. 165-166
3. Brown, Richard. *Histologic Preparations: Common Problems and Their Solutions*. Northfield, III: College of American Pathologist, 2009. 95-101.

PRODUCT INFORMATION

CAT. #	DESCRIPTION
6266-16	Masson's Trichrome Kit

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