

ORCEIN STAIN METHOD FOR HEPATITIS B SURFACE ANTIGEN

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of Hepatitis B antigen.
PRINCIPLE:	Potassium Permanganate oxidizes the tissue providing the binding sites for the stain. Oxalic Acid removes the Permanganate. Orcein stains all tissue components but has a greater affinity for the antigen and so differentiation leaves the antigen distinguishable from the background.
CONTROL:	Tissue known positive for the antigen <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs015.</i>
SPECIMEN PREPARATION:	For demonstration of the Hepatitis B surface antigen, use 4% Neutral Buffered Formalin fixed, paraffin embedded sections cut at 6-10 micrometers For all others any fixation can be used, fixed, paraffin embedded sections cut at 6-10 micrometers
SOLUTIONS:	1. Potassium Permanganate Acidified Set Item# s2037 Potassium Permanganate 0.3% Aqueous..... Solution A Sulfuric Acid 0.6% Aqueous..... Solution B 2. Oxalic Acid 1.5% Aqueous Item# s2034 3. Orcein Solution 1% pH 1.0–2.0 Item# s2033 <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	<u>Potassium Permanganate Acidified Working Solution:</u> Make just before use. Solution A0.3 mL Solution B.....50 mL Distilled water 5 mL
REFERENCE:	Shikata, T. et al. "Staining Methods of Australian Antigen in Paraffin Sections". <i>Jap. J. of Exp. Med.</i> 44:26-34.1974.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to water.
2. Oxidize in Potassium Permanganate Acidified Working Solution for 10 minutes.
3. Rinse in distilled water for 5 minutes.
4. Bleach in Oxalic Acid 1.5% Aqueous for 10 minutes.
5. Stain in Orcein Solution 1% pH 1.0-2.0 for approximately 4 hours.
6. Differentiate quickly in Absolute Ethyl Alcohol.
7. Clear in Xylene, 3 changes, 2 minutes each.
8. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Orcein-positive material localized in cytoplasm of the hepatocytesDark Brown
Elastic FiberBrown

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