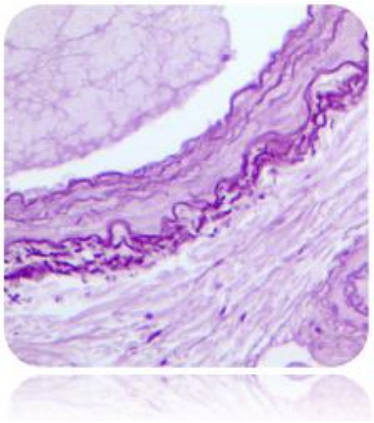
Orcein Stain Kit
(For Hepatitis B and Elastic Fibers)

Description: The Orcein Stain may be used in histology procedures for the visualization of Hepatitis B surface Antigen (HBsAg), elastic fibers, and copper associated proteins. HBsAg appears as irregular shaped aggregates in the cytoplasmic region of the cells. This reagent may be used on formalin-fixed, paraffin-embedded or frozen sections.

Uses/Limitations: Not to be taken internally. For In-Vitro Diagnostic use only. Histological applications. Do not use if reagents become cloudy. Do not use past expiration date. Use caution when handling reagents. Non-Sterile.

Control Tissue: Known hepatitis positive liver. Lung for elastic fiber. Any well fixed tissue cut 3-5 microns.



Results:

BsAg: Dark Brown/Purple
Elastic Fibers: Dark Brown/Purple
Copper Assoc. Proteins: Dark Purple
Background: Light Reddish/Purple

Kit Contents:

Item #	Kit Contents	Volume	Storage
SSC-PPG030	Potassium Permanganate Sol. (5%)	30 ml	18-25°C
SSC-SAQ030	Sulfuric Acid Solution (3%)	30 ml	18-25°C
SSC-OQB125	Oxalic Acid Solution (2%)	125 ml	18-25°C
SSC-OAA125	Orcein Solution	125 ml	18-25°C
SSC-DSA125	Differentiating Solution	125 ml	18-25°C

For information regarding ordering individual components, please contact us at: 800-442-3573.

Precautions: Avoid contact with skin and eyes.
Harmful if swallowed.
Follow all Federal, State, and local regulations regarding disposal.

Procedure:

Prepare Oxidizing Immediately Prior to Beginning Procedure:

Combine:
- 50 ml Distilled Water
- 5 ml Potassium Permanganate Solution (5%)
- 3 ml Sulfuric Acid Solution (3%)

Mix thoroughly.

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Incubate slide in freshly prepared Oxidizing Solution for 10 minutes.
3. Rinse slide briefly in running tap water followed by 1 dip in distilled water.
4. Incubate slide in Oxalic Acid Solution (2%) for 10 minutes.

Note: Section should be colorless following this step.

5. Rinse slide for 1 minute in running tap water followed by 2 dips in distilled water.
6. Apply adequate Orcein Solution to cover tissue and incubate slide in Orcein Solution for 2 hours.

Note: Check periodically and apply additional stain as needed to avoid drying.

7. Rinse slide in Alcohol, Reagent (70%).
8. Apply Differentiating Solution for 3-5 seconds.
9. Dip slide in Alcohol, Reagent (70%) and check slide microscopically for proper differentiation.

Note: Repeat step 8 if necessary.

10. Dehydrate quickly in 3 changes of absolute alcohol.
11. Clear, and mount in synthetic resin.

References:

1. Deodhar K.P., Tapp E., Scheuer P.J. Orcein staining of Hepatitis B Antigen in paraffin sections of Liver Biopsies. Journal of Clinical Pathology; vol. 28: pages 66-70, 1975.
2. Salaspuro, M., Sipponen, P. Demonstration of an intracellular copper-binding protein by Orcein staining in long-standing cholestatic liver diseases. Gut, 1976, volume 17: pages 787-790.

Lot-to-Lot Validation Form
Orcein Stain Kit for Hepatitis B Catalog: SSK-ORCEIN

Kit Lot Number: _____
Kit Expiration Date: _____
Date Tested: _____
Control Tissue (#) _____
Approved for Use: Y/N _____
Date put into use:

If not approved, corrective actions taken:	

Approved by: _____

Kit Component	**Lot #**
Potassium Permanganate, (5%)	
Sulfuric Acid Sol. (3%)	
Oxalic Acid Sol. (2%)	
Orcein Solution	
Differentiating Solution	

Replacement Component if used	Replacement Date	Lot #	Accepted Y/N	Comments
Potassium Permanganate (5%)				
Sulfuric Acid (3%) Sol.				
Oxalic Acid (2%) Sol.				
Orcein Solution				
Differentiating Solution				
Approved By:				

StatLab is providing this form to assist with reagent lot validation as stated in CLIA'88 Standard 493.1256-For reagent(s), the laboratory must do the following: Check each batch (prepared in-house), lot number (commercially prepared) and shipment of reagents, stains, and identification systems (systems using two or more substrates or two or more reagents, or a combination) when prepared or opened for positive and negative reactivity, if applicable.