



Orcein Staining Kit

(Cat# K1432-30, -125; for Hepatitis B and Elastic Fibers; Store at RT)

I. Introduction:

The Orcein Staining 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.

HBsAg: Dark Brown/Purple

Elastic Fibers: Dark Brown/Purple

Copper Assoc. Proteins: Dark Purple

Background: Light Reddish/Purple

II. Application:

- Histological applications
- For *in vitro* diagnostic use

III. Sample Type:

- Any well fixed tissue sections (3-5 microns)
- Control Tissue: known hepatitis positive liver; Lung for elastic fiber.

IV. Kit Contents:

Components	K1432-30	K1432-125	Part Number	Storage Temperature
Potassium Permanganate Sol. (5%)	30 ml	30 ml	K1432-XX-1	RT
Sulfuric Acid Solution (3%)	30 ml	30 ml	K1432-XX-2	RT
Oxalic Acid Solution (2%)	30 ml	125 ml	K1432-XX-3	RT
Orcein Solution	2 x 30 ml	125 ml	K1432-XX-4	RT
Differentiating Solution	30 ml	125 ml	K1432-XX-5	RT

V. User Supplied Reagents and Equipment:

- Distilled water
- Coplin jars
- Forceps
- Absolute alcohol
- Synthetic resin

VI. Shipment and Storage:

All the reagents are shipped and stored at room temperature.

VII. Reagent Preparation:

- Do not use if reagents become cloudy.
- Do not use past expiration date.
- Use caution when handling reagents.
- Non-Sterile

VIII. Procedure (Standard):

Prepare Oxidizing Immediately Prior to Beginning Procedure:

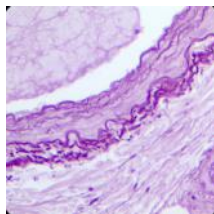
Combine: **50 ml Distilled Water**

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.

IX. Data:



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