ScyTek LABORATORIES INC.

Instructions For Use

HBK-IFU

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Orcein Stain Kit

(For Hepatitis B and Elastic Fibers)

Description and Principle

The Orcein Stain is intended for use in histological demonstration of Hepatitis B surface Antigen (HBsAg), elastic fibers, and copper deposits. HBsAg appears as irregular shaped aggregates in the cytoplasmic region of the cells. This reagent may be used on formalin-fixed, paraffin-embedded sections.

Staining by Orcein relies on oxidation of sulfur containing proteins by potassium permanganate to form sulphonate residues with which orcein can react.

Expected Results

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

Kit Contents

	Storage
1. Potassium Permanganate Sol. (5%)	18-25°C
2. Sulfuric Acid Solution (3%)	18-25°C
3. Oxalic Acid Solution (2%)	18-25°C
4. Orcein Solution	18-25°C
5. Differentiating Solution	18-25°C

Suggested Controls *(not provided)*

Known hepatitis positive liver, Lung for elastic fiber.

Uses/Limitations

For In-Vitro Diagnostic use only.
Do not use if reagents become cloudy or precipitate
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile
Intended for FFPE sections cut at 5-10µm.
This procedure has not been optimized for frozen sections.
Frozen sections may require protocol modification.

Storage

Store kit and all components at room temperature (18-25°C).

Safety and Precautions

Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements.

Procedure:

Prepare Oxidizing Immediately Prior to Beginning Procedure:

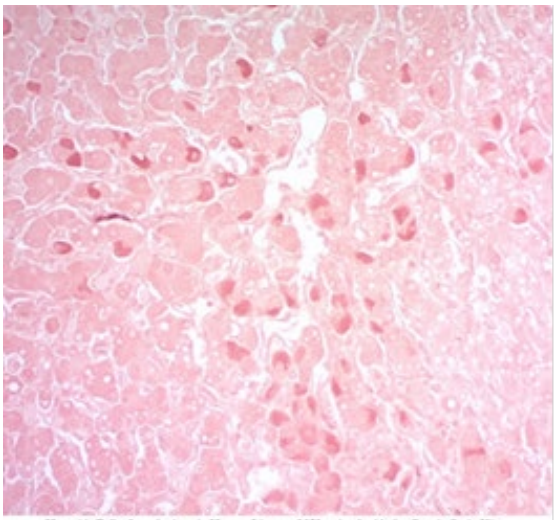
Combine:	50 ml	Distilled Water
	5 ml	Potassium Permanganate Solution (5%)
	3 ml	Sulfuric Acid Solution (3%)

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.



Hepatitis B Surface Antigen in Human Liver at 20X stained with the Orcein Stain Kit.

4. Incubate slide in Oxalic Acid Solution (2%) for 10 minutes or until clear.
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. Incubate slide in coplin jar containing Orcein solution for 4-8 hours (2 hours is sufficient for elastin). Note: Ensure tissue is fully immersed in staining jar. Close lid to prevent evaporation.

7. Rinse slide in Alcohol, Reagent (70%).

8. Differentiate in Differentiating Solution for 10-60 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.

Note: If darker staining is preferred:
1) Incubation time in Orcein solution may be increased.
and/or
2) Differentiation may be omitted by replacing steps 7-9 with a simple rinse with deionized water.

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.

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