

# medilines

## Copper Colour

### for Copper Associated Proteins

#### DESIGN TO DEMONSTRATE

abnormal increased deposits of copper found in the cytoplasm of cells bound to copper-associated protein. Increased amounts of copper are found in the liver in several disease states including Wilson's disease. The excess copper has a toxic effect causing liver damage ranging from acute hepatitis and advanced cirrhosis. In Wilson disease, copper accumulates in certain organs, such as the liver, brain and the cornea of the eye, causing scarring and cirrhosis. Other disease states, which cause an excess of copper in the liver, are chronic biliary obstruction and chronic hepatitis. Copper is normally found in small amounts in the body.

#### REAGENTS & PREPARATIONS

**Copper Solution A**

**Copper Solution B**

**Copper Solution 1:**

**Copper Solution 2:**

**Copper Solution 3:**

Mix 0.5ml A and 7.5ml B. Prepare fresh

Ready to use.

Ready to use.

#### TISSUE SAMPLE

5-6 $\mu$  paraffin sections of 10% neutral buffered formalin.

#### STAINING PROCEDURES

1. Remove Paraffin through xylene, rehydrate through descending graded ethanol and bring sections to distilled water just prior to staining.
2. Place slides into **Copper Solution 1** at 60°C in an oven for 1 hour or at 37°C for 18 hours.

**Or** using plastic Coplin Jars; preheat the **Copper Solution 1** in 1200-watt microwave on half power 30 seconds then incubate for 5 minutes. Do not leave unattended. (Adjust time accordingly for microwave ovens with other wattage. Solution should be heated to about 80°C).

3. Rinse in distilled water, 3-5 changes 5-10 seconds each
4. Place into **Copper Solution 2** for 1-2 minutes.
5. Wash with distilled water.
6. Rinse in **Copper Solution 3** for 15 seconds and rinse in distilled water, 3-4 changes
7. Dehydrate up through graded ethanol to absolute ethanol.
8. Clear in xylene and mount with a resinous medium.

## Expected results

- |                   |                |
|-------------------|----------------|
| ➤ Copper deposits | – Orange/red   |
| ➤ Bile            | – Green/Yellow |
| ➤ Nuclei          | – Blue         |

## Notes

1. During incubation Stain Solutions must be covered.
2. Avoid fixatives with heavy metals (mercury, zinc), as possible false-negative staining have been reported
3. Care must be taken not to over stain with Copper Solution 2, which will mask the positive staining of the copper.
4. Once a satisfactory stain has been achieved, the time should remain constant. Any change in fixation, processing or section thickness warrants reviewing the staining time.
5. Avoid microbial contamination of reagents; otherwise increase in nonspecific staining may occur.
6. The user must validate any changes made to the factory-released procedure.
7. Store reagent packs in an upright position when not in use.
8. Keep reagent packs out of direct sunlight and away from heat-generating sources.
9. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.

## Quality Control

Section of tissue with copper

## Reference

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