
K-673, NISSL STAINING WITH CRESYL VIOLET

Fixation: Most fixatives are satisfactory.

Sections: Paraffin, celloidin, or frozen.

STAINING:

1. Paraffin Sections:

- A. Deparaffinize and hydrate to distilled water.
- B. Stain in working dye solution for 20 minutes. Prepare working solution by mixing 5 mls of stock dye and 100 ml of **Walpole Buffer** (K-673-2). Use solution once and discard.
- C. Rinse quickly in 50%, 70%, and 95% ethyl alcohol. Place in two changes, 2 minutes each of n-butyl alcohol.
- D. Clear in **Xylene** (C-120). Examine under microscope, If the nucleoplasm of neurons is not as colorless as the white matter, return to n-butyl alcohol, 95% ethyl alcohol, and two changes of n-butyl alcohol. If stain is too weak, repeat step C and restain.
- E. Mount.

2. Celloidin Sections:

- A. Wash in distilled water.
- B. Stain for 20 minutes in working dye solution. Prepare working solution by mixing 10 mls of stock dye and 100 ml of **Walpole Buffer** (K-673-2). Use solution once and discard.
- C. Differentiate through 50%, 70% and 95% ethyl alcohol, and n-butyl alcohol. Check differentiation microscopically. Nucleoplasm of neurons should be colorless or faintly blue.
- D. Dehydrate for 12-16 hours in **Terpineol-Xylene** (C-126)
- E. Clear in **Xylene** (C-120). Mount.

3. Frozen Sections:

- A. Mount from water on slides and treat as paraffin sections.
- B. Stain 20 minutes in working dye solution. Prepare working solution by mixing 10 ml of stock dye with 100 ml of **Walpole Buffer** (K-673-2). Use once and discard.
- C. Differentiate rapidly through 50%, 70%, and 95% ethyl alcohol. Place in two changes of n-butyl alcohol, 4 minutes each.
- D. Check differentiation microscopically.
- E. If sections are thick dehydrate for 12-16 hours in **Terpineol-Xylene** (C-126).
- F. Clear in **Xylene** (C-120). Mount.

Stain Results:

Nissl granules	Purple
Nuclei	Blue

References:

Powers, M., and Clark, G., *Stain Techn.*, 38:289, 1955.

Clark, G.: *Staining Procedures*, Williams and Wilkins Co., Baltimore, 3rd Ed., c. 1973, p. 87.