K-680, PAL-WEIGERT METHOD

Fixation: 10% Formalin (F-111)

Sections: Celloidin, low viscosity nitrocellulose or paraffin; or cut frozen sections.

Staining:
1. Mordant sections for 2-24 hours in Ferric Ammonium Sulfate, 4% (K-680-3). Wash in tap water.

2. Place in the working staining solution for 1-2 hours. Wash for 2-3 minutes in tap water.
   • To prepare working solution mix:
     1 volume of LithiumCarbonate (K-680-1)
     with 9 volumes of Alcoholic Hematoxylin, 10% (K-680-2)

3. Decolorize partially in Ferric Ammonium Sulfate, 4% (K-680-3). Continue until the gray and white matter are barely distinguishable. Wash 2-3 minutes in tap water.

4. Differentiate in Potassium Permanganate, 0.4% until gray and white matter are clearly distinguishable when sections are held up to the light. Sections are colored brown by this solution. Rinse quickly in tap water.

5. Complete the decolorization in equal parts of Oxalic Acid, 1% (K-680-5) and Sodium Sulfite, 1% (K-680-6), mixed immediately before using. The gray matter should become completely clear and colorless (except where it contains some myelinated fibers) Wash 2-3 minutes in tap water.

6. Wash 5 minutes or more in Lithium Carbonate, 0.01% (K-680-1) to restore blue color lost in decolorizing. Wash thoroughly in tap water.

7. Counterstain if desired.

8. Dehydrate, clear and mount.

Staining Results:

<table>
<thead>
<tr>
<th>Myelin Sheaths</th>
<th>Dark blue</th>
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<tbody>
<tr>
<td>Other structures</td>
<td>Unstained unless counterstain has been used.</td>
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References: