

**LEDER'S STAIN METHOD FOR ENZYME ACTIVITY**

<b>PURPOSE:</b>	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of esterase activity.
<b>PRINCIPLE:</b>	Esterase activity within the tissue will bind the dye complex, uncomplexed dye is then washed away leaving sites of enzyme activity stained red.
<b>CONTROL:</b>	Skin <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs008.</i>
<b>SPECIMEN PREPARATION:</b>	Fresh smears, Formal Acetone fixed, paraffin embedded sections cut at 5 micrometers
<b>SOLUTIONS:</b>	1. Formal Acetone Solution Item# s2509 2. Sodium Nitrite 4% Solution Item# s2510 3. New Fuchsin Acid Solution Item# s2511 4. Naphthol ASD-Chloroacetate Item# s2512 5. Potassium Phosphate Monobasic 0.9% Aqueous Item# s2513 6. Sodium Phosphate Dibasic 0.44% Aqueous Item# s2514  <i>Solutions can be purchased separately from Poly Scientific.</i>
<b>NOTES:</b>	A counterstain with Harris Hematoxylin can be done after step 5 if desired. The esterase is inhibited by mercury, acids, heat and iodine. False results can occur.  The decal is EDTA.
<b>REFERENCE:</b>	Leder, L.D. "The Selective Enzo-chemical Demonstration of Neutrophilic Myeloid Cells and Tissue Mast Cells in Paraffin Sections". <i>Klin. Wochenschr.</i> 1964. 42:533.

**STAINING PROCEDURE:**

1. Fix smears in cold Formal Acetone for 30 seconds.
2. Deparaffinize and hydrate to distilled water.
3. Wash well with distilled water and air dry.
4. Prepare Hexazotization Solution: Prepare 1 minute before use.  
New Fuchsin Acid Solution ..... 1 mL  
Sodium Nitrite 4% Solution..... 1 mL
5. Prepare Medium Solution: DO NOT FILTER.  
Hexazotization Solution .....0.2 mL  
Naphthol ASD-Chloroacetate .....2 mL  
Potassium Phosphate Monobasic 0.9% Aqueous .....3.8 mL  
Sodium Phosphate Dibasic 0.44% Aqueous ..... 34.2 mL
6. Stain in Medium for 10 minutes. Stain for 1 hour if paraffin embedded.
7. Wash well in water and air dry.
8. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

**RESULTS:**

Enzyme Activity ..... Bright Red

*Poly Scientific R&D Corp.*

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