## **SLIDDER'S REAGENT METHOD FOR PITUITARY**

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of pituitary morphology.
PRINCIPLE:	This stain is very similar to principle trichrome techniques where small molecule dyes are used to stain less porous tissue followed by larger molecule dyes to stain looser textured structures.
CONTROL:	Pituitary
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 4 micrometers
SOLUTIONS:	<ol> <li>Celestine Blue Iron Solution Item# s2494</li> <li>Harris Hematoxylin Item# s212</li> <li>Hydrochloric Acid 1% Aqueous Item# s2207</li> <li>Orange G Phosphotungstic Acid Solution Item# s2495</li> <li>Acid Fuchsin 1% Solution Item# s2438</li> <li>Phosphotungstic Acid 1% Aqueous Item# s2017</li> <li>Fast Green Substitute for Light Green 1% Aqueous Item# s230B</li> </ol> Solutions can be purchased separately from Poly Scientific.
NOTES:	
REFERENCE:	Slidder, W. "Orange-Fuchsin-Green Method for Staining Adenohypophysis". <u>J. Path. Bact</u> . 1961. 82: 532-534.

## STAINING PROCEDURE:

- 1. Deparaffinize and hydrate to water.
- 2. Stain in Celestine Blue Iron Solution for 10 minutes.
- 3. Wash well in running water.
- I. Stain in Harris Hematoxylin for 5 minutes.
- 5. Differentiate in Hydrochloric Acid 1% Aqueous for 30 seconds.
- 6. Wash well in water.
- 7. Rinse in 95% Reagent Alcohol.
- 8. Stain in Orange G Phosphotungstic Acid Solution for 2 minutes.
- 9. Rinse in distilled water.
- Stain in Acid Fuchsin 1% Solution for 2-5 minutes. The stain is progressive and should be continued until the basophil cells are strongly colored.
- 11. Rinse in distilled water.
- 12. Place in Phosphotungstic Acid 1% Aqueous for 4 minutes.
- 13. Rinse in distilled water.
- 14. Stain in Fast Green Substitute for Light Green 1% Aqueous for 1-2 minutes.
- 15. Rinse in distilled water to remove excess stain.
- 16. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 2 changes each.
- 17. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

## **RESULTS:**

Nuclei	Black
Acidophilus	Orange-Yellow
Basophils	Magenta Red
Chromophobes	Pale Grayish-Green
Erythrocytes	Yellow
Stoma	Green

Poly Scientific R&D Corp.

Revision: B-18

PAGE 87

