REAGENT ASSEMBLY KIT
ITEM# k008

COLLOIDAL IRON - PAS METHOD FOR ANTERIOR PITUITARY

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of carboxylated and sulfated mucopolysaccharides and glycoproteins.
PRINCIPLE:	The first step in the procedure employs an acidified colloidal solution of Ferric Hydroxide. Acid mucosubstances and certain acidic mucins are the principal substances in a tissue section that absorb the colloidal ferric ions. It is believed that the initial linkage is as an ionic bond of the ferric ion with the free carboxyl group of the protein. Iron bound to tissue substances is then demonstrated by the Prussian blue reaction. On treatment with Potassium Ferrocyanide, the ferric ion forms Ferric Ferrocyanide, or Prussian blue.
CONTROL:	Normal small intestine, umbilical cord *Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs018.*
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	1. Iron Acetic Acid Solution Item# s2020 2. Potassium Ferrocyanide and HCl Solution Set Item# s2019 Working Solution: Mix equal parts just before use. Potassium Ferrocyanide Solution..... 25 mL Hydrochloric Acid Solution.....25 mL 3. Periodic Acid 0.5% Aqueous Item# s1860 4. Schiff Reagent Item# s272 5. Sulfurous Acid Rinse Item# s1901 6. Orange G 0.5% Aqueous Item# s2016 7. Phosphotungstic Acid 1% Aqueous Item# s2017 8. Acetic Acid 1% Aqueous Item# s100 *Solutions can be purchased separately from Poly Scientific.*
NOTES:	
REFERENCE:	Lillie, R.D. Histopathologic Technique and Practical Histochemistry. McGraw-Hill, 3rd Ed., 1965, pp. 206-207.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to water.
2. Place in Iron Acetic Acid Solution for 10 minutes.
3. Wash well in distilled water.
4. Place in Potassium Ferrocyanide HCl Solution for 5 minutes.
5. Wash well in distilled water.
6. Oxidize for 5 minutes in Periodic Acid 0.5% Aqueous.
7. Rinse in distilled water.
8. Place in Schiff Reagent for 15 minutes.
9. Rinse 3 minutes in each of 3 changes of Sulfurous Acid Rinse.
10. Wash in running tap water for 10 minutes.
11. Stain for 1 minute in Orange G 0.5% Aqueous.
12. Without rinsing, transfer directly to Phosphotungstic Acid 1% Aqueous for 30 seconds.
13. Rinse in Acetic Acid 1% Aqueous.
14. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 2 changes each.
15. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Alpha Cells ..... Yellow-Orange

Beta Cells..... Rose Red with Granulation

Gamma Cells.....Shades of purple due to combined staining with Iron Ferrocyanide and PAS (lightly colored because either filled with very fine granules or they contain coarse sparsely scattered granules)

Delta Cells..... Dark Blue-Purple

Chromotropes ..... Faintest Gray-Mauve Tint

Poly Scientific R&D Corp.

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PolyRnD.com 70 Cleveland Ave. Bay Shore, NY 11706 Phone: 800.645.5825 Fax: 631.254.0618 Email: CustServ@PolyRnD.com