

## E-323 ALCIAN BLUE METHOD (pH 2.5)

**FIXATION:** 10% Buffered Neutral Formalin (F-113)

**SECTION:** Paraffin, @ 6 microns

### **STAINING PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. Mordant in Acetic Acid, 3%, (E-323-3) for three minutes.
3. Stain in Alcian Blue Solution, (E-323-1) for thirty minutes. Wash in running water for ten minutes and rinse in distilled water.
4. Counterstain in Nuclear Fast Red Solution, (E-323-2) for five minutes. Wash in running water for one minute.
5. Dehydrate in 95% Alcohol, Absolute Alcohol, and clear in Xylene, two changes each.
6. Mount with Permount, (M-18)

### **RESULTS:**

Weakly Acidic Sulfated Mucosubstances.....dark blue  
Hyaluronic Acid.....dark blue  
Sialomucins.....dark blue

Nuclei stain weakly in formalin-fixed tissue if Nuclear Fast Red is not used as a counter-stain.

### **REFERENCES:**

AFIP Manual of Histological Staining Methods, 3<sup>rd</sup> ed., Ed. L. Luna: New York:  
McGraw Hill publications, c. 1968, p. 163.

Lev, R. & Spicer, S.S., J. Histochem. Cytochem., 12:309, 1964