

## **C-206 ALIZARIN RED S FOR CALCIUM DEPOSITS**

**Fixation:** Alcoholic Formalin (F-127).

**Embedded:** Paraffin, ester wax, celloidin or gelatin.

### **PROCEDURE:**

1. Bring sections to Alcohol, 50% (C-206-2) and rinse rapidly in distilled water.
2. Cover sections with Alizarin Red S Solution (C-206-1) and follow the staining by transmitted light, on the stage of a staining microscope. Calcium sites will be covered with a vivid orange-red calcium-alizarin lake within a period of 30 seconds-5 minutes. The end point should be chosen when the deposit is heavy but not too diffuse.
3. Shake of excess stain and blot carefully with filter paper or alternatively drain very thoroughly.
4. Place immediately into Acetone (C-206-3) for 10-20 seconds followed by Acetone-Xylene (C-206-4) for 10-20 seconds.
5. Clear in Xylene and mount in Balsam (M-6), Depex (M-9) or Cedarwood Oil (M-7).

### **Stain Results:**

Calcium Sites	Covered and surrounded by a heavy orange red precipitate which is birefringent between crossed polaroids.
Backgroud	Faint Pink

### **References:**

McGee-Russell, S.M., J. Hisrochem., 6:22-42, 1958.

Clark, G., (ed.), Staining Procedures, 3<sup>rd</sup> ed., Williams and Wilkins, Baltimore, p. 140, c. 1973.