

**VON KOSSA STAINING PROTOCOL**  
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1. Fix cells in 0.05% glutaraldehyde in 1X PBS buffer for 10-15 minutes.
2. Wash twice with 1X PBS buffer.
3. Stain in 3% aqueous silver nitrate for 30-60 minutes or until calcium salts become black. Make sure the samples are exposed to a 100 watt desk lamp during staining.
4. Three changes of distilled water.
5. Incubate with 2.5% aqueous sodium thiosulfate for 5 minutes.
6. Wash with water.