

## **Alizarin Red S Staining for Calcium Precipitation (Mineralization)**

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1. Fix cells in 2.5% glutaraldehyde (Freshly prepared in 1x PBS buffer) for 10-15 minutes at room temperature.
2. Remove fixation solution and wash cells with 1x PBS (pH adjusted to 4.2) 1-2 times.
3. Add filtered 2% Alizarin Red Solution to the fixed cells and leave in 37°C incubator for 10-20 minutes (Note: monitor staining under microscope every 2-5 min.).
4. Remove Alizarin Red Solution and rinse cells with PBS 1-2 times.
5. Record staining results with microphotography (positive stained nodules are in orange red).

### **NOTE:**

- 1) Staining should be stable for 1-2 weeks in either dry or wet condition, but the best images are obtained immediately after staining.
- 2) Better staining results are usually achieved by washing the fixed cells with 1x PBS, pH adjusted to 4.2, prior to the staining with Alizarin Red S Solution.

### **2% Alizarin Red S Solution (stable for at least 2-3 weeks)**

Alizarin Red S (Sigma-Aldrich)	2.0 grams
Deionized distilled water	100 ml

Mix well and filter before use.