

Alkaline Phosphatase (Histochemical) Staining for Cultured Cells

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1. Prepare diazonium salt solution: Dissolve a capsule of Fast Blue RR salt (Sigma-Aldrich, Cat # 85L-2, kept at -20°C) in 48 ml distilled water completely at room temperature.
2. Prepare Alkaline-Dye Mixture: Add 2ml Naphthol AS-MX Phosphate Alkaline Solution (Sigma-Aldrich, Cat # 85L-2) to diazonium salt solution.
3. Fix cells with Fixative Solution for 30 seconds at room temperature. Rinse cells carefully with deionized water for 30-60 seconds. Do not let samples dry.
4. Incubate samples with Alkaline-Dye Mixture at room temperature for 10-60 minutes (monitor the staining under microscope every 2-5 minutes). Protect samples from direct light by wrapping plates/flasks with aluminum foil.
5. Discard Alkaline-Dye Mixture upon the completion of staining. Rinse thoroughly with deionized water for 2 minutes (Note: do not let samples dry if counterstaining is required).
6. **[FOR TISSUE STAINING ONLY]**: Counterstain samples with Mayer's Hematoxylin Solution for 10 minutes. Rinse with deionized water for 3 minutes].
7. Evaluate staining microscopically (positive staining is in purple blue).

NOTE: 1) All reagents (except acetone) are provided by the Sigma kit and prepared freshly prior to use.

2) Staining results are stable under a dry condition for at least 1-2 months.

Citrate Working Solution:

Dilute 2ml Citrate Concentrate Solution (Sigma-Aldrich, Cat # 85L-2) to 100 ml with deionized water.

Fixative Solution:

Mix 2 volumes of Citrate Working Solution to 3 volumes of acetone at room temperature.