



Stain: Alkaline Phosphatase

More Staining Procedures For Plastic Embedded Tissue

**Patsy Ruegg, Metabolic Bone Disease Laboratory of the University of Colorado Health Sciences Center
Denver, Colorado**

INDICATION: Osteoblasts

Alkaline Phosphatase can be done on GMA sections but it is tricky. The enzyme seems to be sensitive to heat so care must be taken not to over heat the blocks during polymerization and not to heat the sections onto the slides. I just let them air dry on silane coated slides over night.

REAGENTS:

Naphthol AS-MX phosphate alkaline phosphate solution, 0.25% (Sigma Cat. No. 85-5)
Fast Blue RR Salt (Sigma Cat. No. FBS 25)

PROCEDURE:

1. Measure 50ml of dH₂O into a beaker. Add 2ml of the alkaline phosphate solution and one capsule of the Fast Blue RR Salt.
2. Mix the solution well on a magnetic stirrer.
3. Pour the solution on to the slides in a coplin jar, without filtering.
4. Place the coplin jar in the refrigerator (4C), with the lid on it, and allow to stain over night.
5. The next day, remove the jar from the fridge and pour the solution into the chemical waste can, and rinse the slides very well with dH₂O.
6. Air dry slides and coverslip with permount.

RESULTS:

Osteoblasts and certain other marrow cells stain purple/blue, BONE is unstained.