Alkaline Phosphatase (AP) Buffer Recipe and Protocol

1. **Make up stock solutions**. Sterile filter all solutions into 50 mL conicals. Wrap conicals in foil to limit light exposure. Store in 4°C refrigerator until needed.

1. 1 M Tris (pH 9.5)

-add 6.06g of Tris to 50 mL of ddH2O

-add HCl in 100μl increments until reaching pH 9.5. This take approximately 600µl.

 b. 50mM MgCl2

-add 0.51g of MgCl2 to 50 mL of ddH2O

 c. 1% Tween-20

 -add 500μl of Tween-20 to 50 mL of ddH2O

 d. 1 M NaCl

 -add 2.92g of NaCl to 50 mL of ddH2O

 e. Sterile filter 50 mL of ddH2O (does not need to be stored at 4°C in foil)

2. **Make up working solution of AP Buffer** the day of the coloration reaction. Work in the laminar flow hood, using a sterile 50 mL conical. Wrap conical in foil to limit light exposure. Add the following components in order, using serological pipets.

 5mL of 1 M Tris

 5mL of 50 mM MgCl2

 5mL of 1% Tween-20

 5mL of 1 M NaCl

 30mL of ddH2O