Determination of *K*M and *v*max for Alkaline Phosphatase

# Solutions to be provided:

Substrate, 0.4 mM *p*-nitrophenyl phosphate in 0.2 M Tris-HCl (pH 8.0)

Buffer, 0.2 M Tris-HCl (pH 8.0)

Enzyme solution, 15 mU/200 µL alkaline phosphatase

Product, 0.00005 M *p*-nitrophenol

# Preparation of solutions used by everyone

1 L of 0.2 M Tris-HCl, pH 8.0 buffer, 0.004 M MgCl2.

 Dilute 16.13 g “Trizma Base” (sigma) and 10.55 g Trizma HCl to 1 L with deionized water. Add 0.381 g MgCl2. Mix well.

50 mL 15 mU/0.2 mL alkaline phosphatase

Dissolve 3.75 µL of enzyme in glycerol in 50 mL of tris buffer, pH 8.0.

Alkaline phosphatase from calf intestine: Fisher BP3217-1, $78.77 for 1000 U, 1 U/µL. The volume needed increases the longer the enzyme is in the freezer at -20°C.

250 mL 0.4 mM *p*-nitrophenyl phosphate in 0.2 M tris, pH 8.0

 Place 0.0461 g of *p*-nitrophenyl phosphate di(tris) in a 250 mL volumetric flask. Dilute to the mark with the 0.2 M tris buffer (pH 8.0).

1 L of 0.05 mM *p*-nitrophenol in 0.2 M tris buffer, pH 8.0

 Dilute 16.13 g “Trizma Base” (sigma), 10.55 g Trizma HCl, and 6.96 mg *p*-nitrophenol (139.11 g/mol) to 1 L with deionized water.

# Equipment per pair of students

Spec 20

Kimwipes

6 cuvettes

Rack to hold cuvettes

Parafilm

Many 5 mL autopipetes

# Other Equipment

At least four computers with the enzyme kinetics spreadsheet on the hard drive, and with the capability to print. Excel must have the Solver addin installed; it is part of Excel, so go to File\Options\Add-Ins, and click the “Go” button.