

Assay for Acid Phosphatase Activity at pH 7.2

The assay utilizes p-nitrophenylphosphate (pNPP) as the substrate for the enzyme. One of the products of hydrolysis is p-nitrophenol, which has a pKa value of 7.2. This means that if the assay is performed at pH 7.2, half of this product will be ionized to the p-nitrophenolate ion, which has an absorbance maximum of 405 nm with a molar absorptivity value of $1.8 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. Note that this means that at this pH twice as many moles of phosphate ions (P_i) will be produced as p-nitrophenolate ions! The assay requires the use of a mixture of solutes listed below in addition to the sample volume being evaluated for enzyme content. Note that each assay requires a total volume of 3.00mL.

Assay components:

100 mM Tris HCl

5 mM magnesium chloride

5 mM pNPP

The volume of sample required for the assay depends on the amount of enzyme present in the solution being evaluated. This can be estimated in the following way. First, assume that the total volume of the sample assayed is 100 μL . This means that all other components add up to a total volume of 2.900 mL, and that the difference between the volumes of the enzyme-containing solution you wish to assay (see below) and 100 μL will consist of 0.2 M ammonium sulfate containing 1 mM EDTA. The fraction of the sample volume that actually consists of the enzyme-containing solution will vary, but can be approximately calculated by assuming that each of the fractions evaluated has the same protein content and specific activity as indicated in the Table below. The total activity (calculated by multiplying protein content by specific activity) corresponds to that obtained under acidic conditions. The activity at pH 7.2 can be extrapolated by dividing this number by a factor of 2.5, the typical factor by which the activity decreases from optimal conditions as the pH is increased to this value. Using this information and a target rate of 0.2 AU/min for the increase in A_{405} , calculate the number of μL of enzyme-containing solution needed. Note that under these conditions the enzyme is saturated with substrate and will be operating at V_{max} for at least ten minutes. A unit of activity is defined as 1 $\mu\text{mol P}_i/\text{min}$. Try the calculated volume of solution for the assay, and make adjustments (as needed) to achieve the target rate ($\pm 0.05 \text{ AU/min}$).

Sample Data Set for the Isolation of Acid Phosphatase:

Fraction	V_T (mL)	Total Protein (mg)	Spec. Act. (units/mg)
I	165	7324	0.08
II	59	406	0.87
III	26	35	7.2
IV	17	5.4	22.2

This Table will be extended later to include total acidic activity, estimated total activity at pH 7.2 and percent recovery.