QuantiChrom™ Acid Phosphatase Assay Kit (DACP-100)

Quantitative Colorimetric Kinetic Acid Phosphatase Activity Determination

DESCRIPTION

Acid Phosphatase (ACP) is an enzyme which catalyzes the cleavage of phosphate groups from other molecules during digestion. Acid phosphatase can be found in lysosomes and become active after fusing with endosomes, acidifying the pH and, thus, creating an optimal environment for ACP. ACP can also be found in bone, spleen, liver, kidney and blood. Serum levels can be used as a biomarker for prostatic carcinoma, although prostate-specific antigen (PSA) is more widely used.

BioAssay Systems' non-radioactive, colorimetric ACP assay is based on the cleavage of *p*-nitrophenol from the synthetic substrate. *p*-Nitrophenol becomes intensely colored after addition of the stop reagent. The increase in absorbance at 405 nm after addition of the stop reagent is directly proportional to the enzyme activity.

KEY FEATURES

Fast and sensitive. Linear detection range (20 µL sample): 0.05 to 60 U/L for a 30 minute reaction.

High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

Acid Phosphatase activity determination in biological samples (e.g. plasma, serum, cell lysate, tissue samples.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	12 mL	**pNPP Liquid:**	280 µL
Stop Reagent:	12 mL	**Standard:**	1 mL

Storage conditions. The kit is shipped at room temperature. Store the Standard and stop reagent at 4°C and all other reagents at -20°C. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Substrate and Stop Reagent to samples should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Sample Preparation: Serum and plasma should be diluted 2-5 fold.

Tissue: Prior to dissection, rinse tissue in Tris buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL 50 mM Tris buffer (pH 7.5). Centrifuge at 14,000 x g for 10 min at 4°C. Remove supernatant for assay.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold 50 mM Tris buffer (pH 7.5), approximately one million cells per mL. Centrifuge at 14,000 x g for 10 min at 4°C. Remove supernatant for assay.

All samples can be stored at –80 to –20°C for at least one month.

Reagent Preparation: Equilibrate all components to desired reaction temperature (e.g. 25°C or 37°C).

Standard Preparation:

Mix 20 µL of 12.5 mM Nitrophenol standard with 230 µL dH_2O to make 1000 µM Premix.

No	Premix + dH_2O	Vol (µL)	Nitrophenol (µM)
1	100 µL + 0 µL	100	1000
2	60 µL + 40 µL	100	600
3	30 µL + 70 µL	100	300
4	0 µL + 100 µL	100	0

Reaction Preparation:

1. Transfer 20 µL of each sample into separate wells. Transfer 20 µL of each standard (OD_{STD}) into wells of a clear flat bottom 96-well plate.
2. The Working Reagent is prepared by mixing together for each well 85 µL of assay buffer and 2 µL of pNPP Liquid. Add 80 µL of Working Reagent to all standard and sample wells. Tap plate briefly to mix.
3. Incubate at 25°C or desired temperature for 30 minutes. Add 50 µL of Stop Reagent to each well. Tap plate briefly to mix.
4. Read OD_{405nm}.

Note: If your sample is colored or opaque, then a sample blank (OD_{BLANK}) will be needed. Add 20 µL of sample to a well, and add 80 µL of dH_2O. After incubation add 50 µL Stop Reagent.

CALCULATION

Subtract blank OD (water, #4) from the standard OD values and plot the ΔOD against standard concentrations. Determine the Slope and use the following equation to calculate Acid Phosphatase activity.

$$\textbf{ACP Activity} = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Time} \bullet \text{Slope}} \times n \quad (U/L)$$

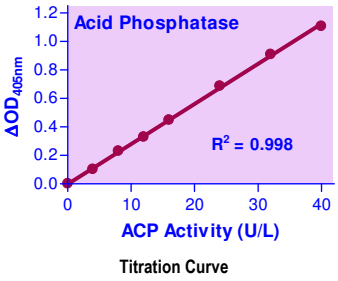
where OD_{SAMPLE} is the OD_{405nm} value for each sample and OD_{BLANK} is the OD_{405nm} value of the water (standard #4) or the sample blank if one was used. Slope is the slope of the linear regression fit of the standard points and Time is the reaction time (30 min). *n* is the dilution factor.

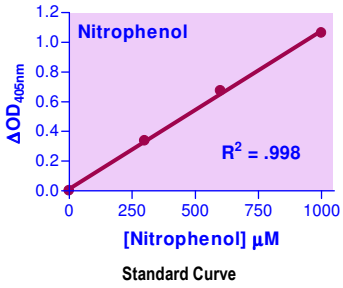
Unit definition: 1 Unit (U) of ACP will catalyze the conversion of 1 µmole of p-Nitrophenyl phosphate to p-Nitrophenol and phosphate per min at 25°C and pH 5.3.

Note: If sample ACP activity exceeds 60 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with ACP activity < 1 U/L, the incubation time can be extended up to 60 minutes for greater sensitivity.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.





LITERATURE

1. Henneberry, M.O.; Engel, G.; Grayhack, J.T. (October 1979). "Acid Phosphatase". *The Urologic clinics of North America* 6 (3): 629–41.
2. Bull, H.; Murray, P.G.; Thomas, D.; Fraser, A.M.; Nelson, P.N. (April 2002). "Acid Phosphatases". *Molecular Pathology* 55 (2): 65–72. Retrieved 11 May 2015.
3. Taira A, Merrick G, Wallner K, Dattoli M (July 2007). "Reviving the acid phosphatase test for prostate cancer". *Oncology (Williston Park, N.Y.)* 21 (8): 1003–10.