Technical Manual

FluoroDazzle Acid Phosphatase Activity Assay Kit

Catalogue Code: BA0156

Pack Size: 100 assays

Research Use Only

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.

The Assay Genie non-radioactive, fluorimetric FluoroDazzle Acid Phosphatase Activity Assay Kit is based on the cleavage of a synthetic substrate. The product methylumbelliferone (MUB) becomes intensely fluorescent after addition of the stop reagent. The increase in fluorescence at 360/450 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): 0.008 to 10 U/L for a 30 minute reaction.

Convenient and 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	**MUP Substrate:**	120 µL
Stop Reagent:	12 mL	**Standard:**	120 µL

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receiving. 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 × 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 × 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 25 µL of Standard (MUB) with 475 µL dH_2O to make 100 µM Premix.

No	Premix + dH_2O	Vol (µL)	MUB (µM)
1	100 µL + 0 µL	100	100
2	60 µL + 40 µL	100	60
3	30 µL + 70 µL	100	30
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 into wells of a black flat-bottom 96-well plate.
2. The Working Reagent is prepared by mixing together for each well 85 µL of assay buffer and 1 µL of MUP Substrate. 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 Fluorescence at 360/450nm.

CALCULATION

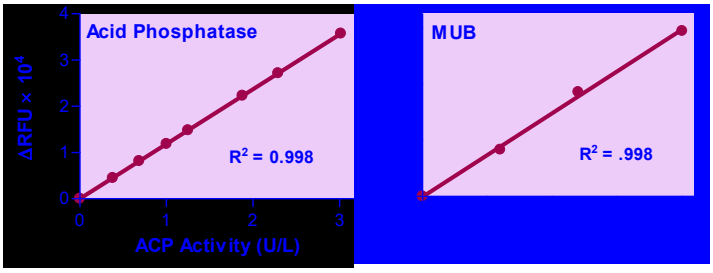
Subtract blank F (water, #4) from the standard F values and plot the ΔF against standard concentrations. Determine the Slope and use the following equation to calculate Acid Phosphatase activity.

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

where FSAMPLE is the RFU value for each sample and F_{BLANK} is the RFU value of the water (standard #4). 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 4-methylumbelliferyl phosphate to 4-methylumbelliferone and phosphate per min at 25°C and pH 5.3.

Note: If sample ACP activity exceeds 10 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with ACP activity < 0.1 U/L, the incubation time can be extended up to 60 minutes for greater sensitivity.



Titration Curve **Standard Curve**

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), black flat-bottom 96-well plates (e.g. VWR cat# 89089-582), 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.

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