Tribioscience

Alkaline Phosphatase (ALP) Fluorometric Assay

High-Sensitive Quantitation for Alkaline phosphatase Activity

Catalog Number	Kit Size
TBS2078-100	100 assays
TBS2078-200	200 assays

DESCRIPTION

Alkaline phosphatase (ALP) Alkaline is a hydrolase enzyme which catalyzes the hydrolysis of phosphate esters in alkaline buffer and produces an organic radical and inorganic phosphate. The changes of ALP level and activity are associated with several diseases in the liver and bones. ALP is also a common enzyme conjugated to secondary antibodies for immunoassay like ELISA and chemiluminescence.

Tribioscience's Alkaline Phosphatase Activity Fluorometric Assay Kit provides a highly sensitive and convenient way for direct detection of ALP activity in different biological samples. In the assay, ALP removes the phosphate group of the non-fluorescent 4-Methylumbelliferyl phosphate disodium salt (4-MUP) substrate producing an intense fluorescent signal (Ex/Em = 360 nm/450 nm). The kit is an ultra-sensitive, HTS-ready assay that is more sensitive than colorimetric methods. The assay is suited for both research and drug discovery.

APPLICATIONS

Direct Assays: Acid phosphatase in serum, plasma, urine, and other bio-samples.

KEY FEATURES

Flexible: Suitable for colorimetric assay.

Accurate: Use 50 µL samples. Detection ranges 0.3-80 U/L in 96-well plate for fluorometric assay.

Simple and high-throughput: Just load-incubate-Read. Kit can be used as a robust method.

Time saving: 20-30 minutes.

KIT CONTENTS

Component	TBS2075-100	TBS2075-200
Assay Buffer	10mL	20 mL
Substrate	6mL	12mL
ALP Standard Stock (200U/L)	120 µL	240 µL
Stop Solution	10 mL	20 mL

STORAGE AND HANDLING

Store kit at -20°C. Shelf life of 2 year. Protect from light.

PRECAUTION

Inhibitors of ALP, such as tartrate, fluoride, EDTA, oxalate, should be avoided in sample preparation.

PROCEDURES

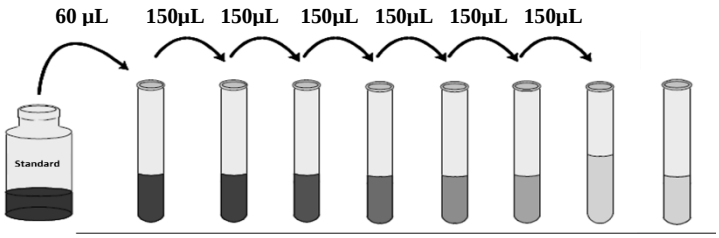
Sample Preparations

Sample Preparations: Serum, plasma, urine, semen, and cell culture media can be assayed directly. Cells (1×10^5) or tissue (~10 mg) can be homogenized in 150 µl Assay Buffer, centrifuge to remove insoluble material at 13,000g, 3 minutes. The supernatant can be used as test sample for the assay testing.

Standard Curve Preparations (Fig. 1)

1. Label 1.5mL tube from Std1 to 8. As below the diagram.
2. Add 240 µL of 1x Assay Buffer to Std1, and 150 µL to Std2 to 8.
3. Take 30 µL of 200 U/L ALP Standard Stock solution to Std1, then make 2x series dilution from Std2 through Std7 by transferring 150µL to the next concentration, Std8 is 1x Assay Buffer alone as a standard 0. The standard concentration range is 40, 20, 10, 5, 2.5, 1.25, 0.625 U/L, and 0.

Fig. 1 Diagram for AP Standard Preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	240	150	150	150	150	150	150	150
STD Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition (µL)	60	150	150	150	150	150	150	
Final Conc (U/L)	40	20	10	5	2.5	1.25	0.625	0

Assay Procedures

1. Add 50 µL of standard or sample to each well of a microplate in duplicate manner.
2. Add 50 µL substrate to each well, and incubate at room temperature for 20-60 minutes, protect from light.
3. Add 50 µL Stop Solution to terminate the reaction, and fully mix.
4. Measure fluorescence intensity at (λexc= 360nm, λem = 450nm), in a plater reader.

Data Calculation

Plot the RFU measured for each Standard against the ALP activity. Determine the slope using linear regression fitting.

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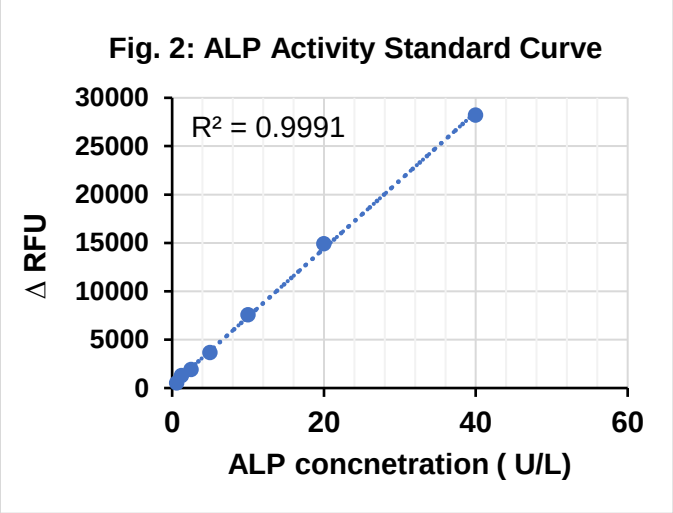
ALP activity of the sample is:

ALP Activity = [(FSAMPLE – FBLANK)/(Slope x t)] x n (U/L

FSAMPLE and FBLANK are fluorescence intensity values of the Sample and the Blank (i.e. no Standard well). t is the reaction time (e.g. 30 min). n is the dilution factor. If the calculated value is higher than 40 U/L, use shorter incubation time, or dilute sample in water and repeat assay. Multiply the result by the dilution factor n. Unit definition: 1 unit (U) of ALP catalyzes the conversion of 1 μmole of 4-methylumbelliferyl phosphate to 4- methylumbelliferone at pH 10.5 and room temperature (25°C).

Typical Standard Curve

Fig.2 displays a typical standard curve of ALP activity. It is only used as a reference.



RELATED PRODUCTS

ATP Colorimetric/Fluorometric Assay (TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
Glucose Oxidase Colorimetric/Fluorometric Assay (TBS2088)
β-Hexosaminidase Activity Assay (TBS2105)
Cytochrome C Oxidase Activity Assay (TBS2115)
Glucose Determination Colorimetric/Fluorometric Assay (TBS2087)
Non-esterified Fatty Acid Assy (TBS2203)
Glycerol Colorimetric / Fluorometric Assy (TBS2204)
Caspase-3 Fluorometric Assay kit (TBS3230)
Tryptase Activity Assay (TBS2101)

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