QuantiFluo™ Alkaline Phosphatase Assay Kit (QFAP-100)

Fluorimetric Determination of Alkaline Phosphatase Activity at 360/450 nm

DESCRIPTION

Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in an alkaline environment, resulting in the formation of an organic radical and inorganic phosphate. In mammals, this enzyme is found mainly in the liver and bones. Marked increase in serum ALP levels, a disease known as hyperalkalinephosphatasemia, has been associated with malignant biliary obstruction, primary biliary cirrhosis, primary sclerosing cholangitis, hepatic lymphoma and sarcoidosis.

Simple, direct and automation-ready procedures for measuring ALP activity in serum are becoming popular in Research and Drug Discovery. This improved method utilizes 4-methylumbelliferyl phosphate that is hydrolyzed by ALP into a highly fluorescent product 4-methylumbelliferone. The rate of the fluorescence increase is directly proportional to the enzyme activity.

4-Methylumbelliferyl phosphate

↓ *Alkaline Phosphatase*

4-Methylumbelliferone + phosphate

fluorescent (360/450 nm)

KEY FEATURES

High sensitivity and wide linear range. Use 10 µL sample. Detection limit of 0.02 U/L (20 min reaction).

Homogeneous and simple procedure. Simple "mix-and-measure" procedure allows reliable quantitation of ALP activity within 20 minutes.

Robust and amenable to HTS. All reagents are compatible with high-throughput liquid handling instruments.

APPLICATIONS

Direct Assays: ALP activity in serum, plasma and other sources.

Characterization and Quality Control for ALP production.

Drug Discovery: high-throughput screen for ALP modulators.

KIT CONTENTS (100 tests in 96-well plates)

Reagent: 14 mL (pH 10.5) **100× Standard:** 120 µL

Storage conditions. The kit is shipped at room temperature. Store at -20°C. Shelf life of 2 years 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

Thaw reagents to room tempeature prior to use. This assay is based on a kinetic reaction. Use of a multi-channel pipettor is recommended. Addition of Reagent to samples should be quick and mixing should be brief but thorough. Assays can be executed at room temperature or 37°C.

Sample preparation: ALP is stable for 48 hours at 4°C and 2 months at -20°C. EDTA, oxalate, fluoride, citrate are known inhibitors of ALP and should be avoided in sample preparation. Serum, plasma (no EDTA/citrate, ideally unhemolyzed) and cell culture media can be assayed directly.

Procedure using 96-well plate:

1. *Standards*. First mix 5 µL of the provided 100× Standard (2 mM 4-methylumbelliferone) with 495 µL distilled water to obtain 1× Standard (20 µM 4-methylumbelliferone). Prepare standards as shown in the Table below.

No	1× Standard + H_2O	Vol (µL)	4-Methylumbelliferone (µM)
1	100 µL + 0 µL	100	20
2	60 µL + 40 µL	100	12
3	30 µL + 70 µL	100	6
4	0 µL + 100 µL	100	0

Transfer 10 µL of each Standard and each Sample to separate wells of the plate.

2. Using a multi-channel pipettor, add 90 µL Reagent to all Standard and Sample wells. Quickly tap plate to mix and incubate for a desired period of time (e.g. 20 min) at desired temperature (e.g. 25°C).
3. Read fluorescence intensity (λ_{exc}= 360 nm, λ_{em} = 450 nm) on a plate reader.
4. *Calculation*. Plot the RFU measured at 20 min for each Standard against the 4-methylumbelliferone concentration. Determine the slope using linear regression fitting. ALP activity of the sample is

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

where F_{SAMPLE} and F_{BLANK} are the fluorescence intensity values of the Sample and the Blank (i.e. no Standard well) respectively. t is the reaction time (e.g. 20 min). n is the dilution factor. If the calculated value is higher than 1 U/L, use a 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 per minute at pH 10.5 and room temperature (25°C).

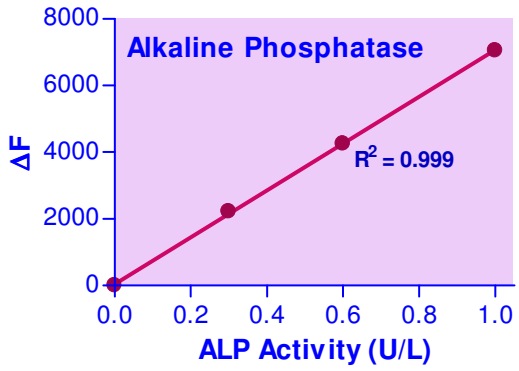
MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories (e.g. multi-channel pipettor).

Black flat-bottom 96-well plates (e.g. Corning Costar) and plate reader.

GENERAL CONSIDERATIONS

1. For low ALP activity samples (< 1 U/L), it is recommended to prolong the incubation time to for example 60 min.
2. The reaction volumes can be scaled down for 384-well assay or up for cuvette assays.



Standard Curve (20 min incubation)

PUBLICATIONS

1. Domazetovic, V., et al (2020). Blueberry juice antioxidants protect osteogenic activity against oxidative stress and improve long-term activation of the mineralization process in human osteoblast-like saos-2 cells: Involvement of sirt1. Antioxidants (Basel, Switzerland), 9(2).
2. Laura, BP et al. (2015). Lasting effects of butyrate and low FM/FO diets on growth performance, blood haematology/biochemistry and molecular growth-related markers in gilthead sea bream (Sparus aurata). Aquaculture. 454 (2016) 8-18.
3. Ballester-Lozano GF et al. (2015). Comprehensive biometric, biochemical and histopathological assessment of nutrient deficiencies in gilthead sea bream fed semi-purified diets. Br J Nutr. 114(5):713-26.