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Technical Datasheet

EZAssay™ Alkaline Phosphatase Activity Estimation Kit

Product Code: CCK035

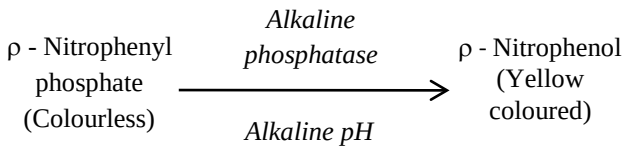
1. Introduction

Alkaline phosphatase (ALP) is a group of isoenzymes that hydrolyze phosphate esters and generate inorganic phosphate at an alkaline pH. ALP is present in all mammalian tissues, and multiple isozymes are differentially distributed in the body. Change in alkaline phophatase activity is associated with various disease states. ALP elevation also serves as an osteogenic differentiation marker.

EZAssay™ Alkaline Phosphatase Activity Estimation Kit is a simple colorimetric kit designed to detect the presence of alkaline phosphatase activity.

2. About the kit

Alkaline phosphatase catalyzes the hydrolysis of colourless ρ – Nitrophenyl phosphate (ρNPP) to yellow coloured ρ – Nitrophenol (ρNP). The absorbance ρ – Nitrophenol can be measured at 405nm. The rate of increased absorbance at 405nm is proportional to the enzyme activity.



Unique feature of this reaction is that the other phosphatases present in the serum do not interfere with the estimation because reaction pH is alkaline and is highly specific for alkaline phosphatase activity.

3. Kit Contents

The kit is sufficient for 100 assays in 1ml cuvettes including controls, blank and samples.

Contents		Quantity	Storage
Code	**Description**		
CCK035(A)	Assay Buffer	100ml	2-8°C
CCK035(B)	Alkaline phosphatase substrate	1g	2-8°C
CCK035(C)	Alkaline phosphatase standard	5 x 500µl	2-8°C

4. Materials required but not provided in the kit:

- Test sample (plasma or serum or cells lysate)
- Adjustable pipettes and pipette aid
- 1ml Quartz cuvettes
- Spectrophotometer capable of measuring absorbance between 405nm
- Cell culture grade water

5. General Guidelines:

Accuracy

- To obtain statistically significant data, perform the assay in triplicates or more.
- Accuracy of the assay depends on pipetting skills of the personnel. Inappropriate addition and mixing practices may result in erroneous and false-positive or false-negative results.
- Use of a repeating pipettor is recommended to pipette reagents. This saves time and helps maintain more precise incubation times.
- Pipette tip should be equilibrated with the reagent before use. This is carried out by slowly filling up the tip with reagent and gently expelling the contents several times.

- Care should be taken so that no bubbles are introduced into the cuvette during pipetting or mixing of the reagents.

Procedural precautions

- Do not leave the reagent bottles and sample bottles open for prolonged duration. Replace the caps immediately after use.

6. Directions for use:

Users are advised to review entire procedure before starting the assay

6.1. Preparation of reagents

Substrate solution

Prepare 167mM solution of ρ – Nitrophenyl phosphate by dissolving 248mg alkaline phosphatase substrate [(CCK035 (B)] in 1ml of cell culture grade water. It is recommended to prepare this solution freshly as per the required quantity. Store the solution in amber coloured bottle at 2 - 8°C until used. The solution remains stable for 12 hours at 2 – 8°C.

6.2. Alkaline Phosphatase Estimation

1. Keep substrate solution, enzyme solution and sample on ice during the assay.
2. Add the appropriate quantities of assay buffer and substrate solution in blank, control and sample tubes. (Refer table 1)
3. Equilibrate the tubes at 37°C by keeping in water bath (5 mins).
4. Add 20µl alkaline phosphatase control solution to control tube and 20µl test sample to test tube.
5. Immediately mix the contents by inversion and transfer the content in a 1ml quartz cuvette.
6. Record the increase in absorbance at 405nm up to 5 minutes.
7. Calculate the concentration of alkaline phosphatase using formulae given in section 7.

Table 1: Assay procedure for alkaline phosphatase estimation

	Blank	Control	Test
Assay buffer	980µl	960µl	960µl
Substrate	20µl	20µl	20µl
Equilibrate the tubes to 37°C by keeping in a water bath.			
ALP enzyme	-	20µl	-
Test sample	-	-	20µl

7. Calculations

Alkaline phosphatase (units/ml) =

$$\frac{(\Delta A_{405nm} / \text{min Test} - \Delta A_{405nm} / \text{min Blank}) \times DF \times TV}{18.5 \times EV}$$

Where
DF = Dilution factor
TV = Total volume of the assay (ml)
EV = Volume of the enzyme sample used for assay (ml)
18.5 = Millimolar extinction coefficient of ρNPP at 405nm.

Alkaline phosphatase (units/mg solid) =

$$\frac{\text{Units/ml enzyme}}{\text{Mg solid/ ml enzyme}}$$

Alkaline phosphatase (units/mg protein) =

$$\frac{\text{Units/ml enzyme}}{\text{Mg protein/ ml enzyme}}$$

All units given in this procedure are Diethanolamine units.

Unit definition

Diethanolamine (DEA) units: The amount of enzyme causing the hydrolysis of one micromole of pNPP per minute at pH 9.8 and 37°C (diethanolamine buffer).

Glycine units: The amount of enzyme causing the hydrolysis of one micromole of pNPP per minute at pH 9.6 and 25°C (glycine buffer).

Unit Conversion: One Glycine unit as described above is equivalent to approximately three DEA units. This reaction system is in Glycine buffer.

8. Troubleshooting Tips

Problems	Possible Causes	Recommended Solutions
Random absorbance values / dispersed duplicate and triplicate values	Pipetting errors	Do not splash contents of the tube; equilibrate the pipette tips before pipetting each reagent
	Air bubbles formed in the tube/cuvette	Pipette gently against the wall of the tubes; remove the bubbles by gently tapping the side of the tube/cuvette
	Multiple freezing – thawing of the samples	Aliquot the samples into smaller volumes before freezing to avoid multiple freeze-thaw cycles
	Use of old or inappropriately stored samples	Use fresh samples or store the samples at appropriate temperatures
	Use of partially thawed samples	Thaw the sample completely and mix gently before use
	Prolonged exposure of sample to room temperature	Avoid exposure of the sample to room temperature. Keep the sample on ice bath during assay procedure.
	Use of old substrate solution	Use freshly prepared substrate solution
	Prolonged exposure of substrate solution to light	Store the substrate solution in amber coloured bottle to avoid light exposure
	Use of wrong wavelength	Ensure that the wavelength is 405nm

9. Storage and shelf life

Store the reagents at 2 – 8°C on receipt.

For long term usage, store alkaline phosphatase standard at -20°C.

Use before expiry date given on the product label.