# QuantiChrom™ Alkaline Phosphatase Assay Kit (DALP-250)

## Colorimetric Kinetic Determination of Serum Alkaline Phosphatase Activity

## 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. BioAssay Systems' QuantiChrom™ Alkaline Phosphatase Assay Kit is designed to measure ALP activity directly in biological samples without pretreatment. The improved method utilizes *p*-nitrophenyl phosphate that is hydrolyzed by ALP into a yellow colored product (maximal absorbance at 405nm). The rate of the reaction is directly proportional to the enzyme activity.

*p*-Nitrophenyl phosphate $\xrightarrow{ALP}$ *p*-nitrophenol + phosphate

## KEY FEATURES

**High sensitivity and wide linear range**. Use 5 µL serum or plasma sample. The detection limit is 2 IU/L, linear up to 800 IU/L.
**Homogeneous and simple procedure**. Simple "mix-and-measure" procedure allows reliable quantitation of ALP activity within 5 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 inhibitors and evaluation of ALP inhibitors.

## KIT CONTENTS (250 tests in 96-well plates)

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|---|---|
| **Assay Buffer: 50 mL, pH 10.5** | **Mg Acetate: 1.5 mL 0.2M** |
| **pNPP Liquid: 600 µL 1M** | **Tartrazine Standard: 10 mL 47µM** |

**Storage conditions**. Store all components at 4°C. For long term storage, Keep the pNPP substate at -20°C. Shelf life: 12 months.

**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 Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at room temperature or 37°C.

**Reagent Preparation:** equilibrate reagents to room temperature. The Working Solution is prepared by mixing for each 96-well assay, 200 µL Assay Buffer, 5 µL Mg Acetate (*final* 5 mM) and 2 µL pNPP liquid substrate (10 mM). Fresh reconstitution is recommended, although the Working Solution is stable for at least one day at room temperature.

Specimen: serum and heparinized plasma (no EDTA), ideally unhemolyzed. ALP is stable for 48 hours at 4°C and a few months at -20°C. EDTA, oxalate, fluoride, citrate are known inhibitors of ALP and should be avoided while collecting specimen.

### Procedure using 96-well plate:

1. Transfer 200 µL distilled water ($H_2O$) and 200 µL Tartrazine Standard (Tart) into wells of a clear bottom 96-well plate.
2. Carefully transfer 5 µL samples into other wells.
3. Pipet 195 µL Working Solution quickly to sample wells. The total volume in the sample wells is 200 µL. Tap plate briefly to mix.
4. Read $OD_{405nm}$ ($t$ = 0), and again after 4 min ($t$ = 4 min) on a plate reader. Alternatively, record the kinetics of changes in $OD_{405nm}$.

### Procedure using Cuvette:

1. Transfer 20 µL samples into 1-cm cuvettes.
2. Pipet 1000 µL Working Solution to samples. Mix briefly.
3. Read $OD_{405nm}$ shortly after the mixing, and again after 4 min.

Note: if sample ALP activity exceeds 800 IU/L, dilute samples in saline and repeat the assay, multiply the result by the dilution factor.

## CALCULATION

ALP activity in IU/L of the sample is calculated as

$$= \frac{(OD_{SAMPLE\ 4} - OD_{SAMPLE\ o}) \cdot 1000 \cdot RV}{4 \cdot \varepsilon \cdot l \cdot SV}$$

$OD_{SAMPLE\ 4}$ and $OD_{SAMPLE\ o}$ are $OD_{405nm}$ values of sample at 4 min and 0 min. The factor 1000 converts IU/mL to IU/L. RV is the total reaction volume, i.e, 200 µL for 96-well assay and 1020 µL for cuvette assay. "4" is the incubation time (min). $\varepsilon = 18.75\ mM^{-1} \cdot cm^{-1}$. $l$ (light path, cm) is 1 cm for cuvette assay, and calculated for 96-well assay from the Tartrazine Standard, $l = (OD_{Tart} - OD_{H2O})/(\varepsilon \cdot c) = 1.321 \cdot \Delta OD$. SV is the sample volume, i.e., 5 µL for 96-well assay and 20 µL for the cuvette assay.

**Conversions**: 1 nkat/L (SI Units) = 16.67 x IU/L.

## MATERIALS REQUIRED, BUT NOT PROVIDED

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

### Procedure using 96-well plate:

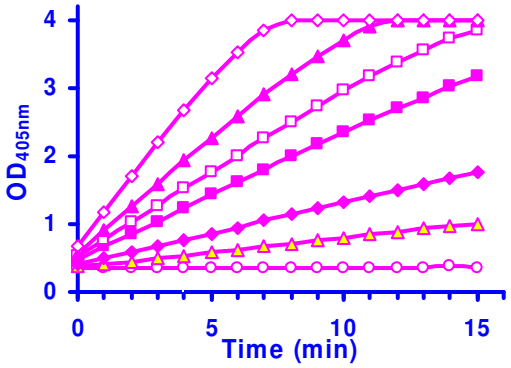
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

### Procedure using cuvette:

Spectrophotometer and cuvets for measuring OD 405nm.

## EXAMPLES

Samples were assayed in duplicate (n = 2) using the 96-well plate protocol. The ALP activity (IU/L) was 13.4 ± 0.4 for a human serum, 190.4 ± 1.6 for rat serum and 202.8 ± 4.3 for goat serum, respectively.




**Kinetics of ALP reaction in 96-well plate assay**
with increasing ALP concentration

## LITERATURE

1. Eaton RH. (1977) Plasma alkaline phosphatase assay: interconversion of results by two methods. *Clin Chem*. 23(11):2148-50.

2. Ambler J, Arnold DF, Green AG (1970). A study of the alkaline phosphatase activity in some commercial quality control sera with p-nitrophenyl phosphate and phenyl phosphate substrates. *Clin Chim Acta*. 27(2):350-3.

3. Williamson T. (1972). A comparison between the phosphastrate and phenyl phosphate methods of alkaline phosphatase assay. *Med Lab Technol*. 29(2):182-7.