Bioway Chemistry Reagent Series

The Serum Alkaline Phosphatase Reagent Kit

Detection of Alkaline Phosphatase in Human Serum or Plasma on Chemistry Analyzers

Cat. No. R005K11 ***The Serum ALP Reagent Kit***

SUMMARY OF TEST PROCEDURE

Figure 1: Procedure Diagram

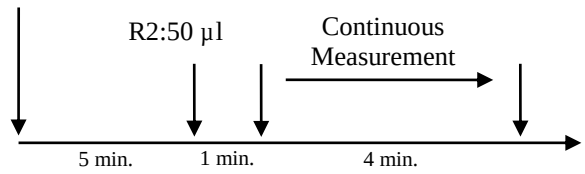

Table 1: Instrument Parameters*

F factor	2713	Slope of reaction	Increase
Testing wavelength	Dλ : 405 nm Sλ : 480 nm	Sample volume	6 µl
Test method	Rate Method	R1 volume	250 µl
Reaction temperature	37℃	R2 volume	50 µl

*Refer to Figure 1 and the package insert for detail

INTENDED USE

Bioway Chemistry Reagent Series **ALP Reagent Kit** (the Kit) is an assay intended for *in vitro* quantitative detection of Alkaline Phosphatase in human serum or plasma on automated clinical chemistry analyzers.

SUMMARY AND EXPLANATION

Alkaline phosphatase can be found in many human organs such as liver, kidney, bone, intestine and placenta. This hydrolyzed enzyme is able to trigger dephosphorylation reactions under alkaline enviroment. There are some physical reasons, like being pregnant or in childhood, to get a high level serum alkaline phosphatase result and it usually makes no problem. But if it is not, the patient can be suspected to have obstructed bile duct or other coeliac diseases. The concentration of serum alkaline phosphatase is decreased in people with pernicious anemia, aplastic anemia, chronic myelogenous leukemia or taking oral contraceptives.

TEST PRINCIPLES

The Kit utilizes enzymatic and kinetic reactions to measure the ALP activity (U/L) in human serum or plasma.

$$\text{4-nitrophenoxide} + H_2O \xrightarrow{\text{ALP}} \text{4-Nonylphenol} + \text{Phosphate}$$

In this method 4-nitrophenoxide is utilized as a substrate to be catalyzed by Alkaline Phosphatase for transphosphorylation reaction. The process is quantified by measuring the absorabances at 405 nm in a kinetic fashion.

The rate of increase in absorbance at 405 nm is directly proportional to the ALP activity in the sample.

MATERIALS PROVIDED

Reagents:

R1	AMP buffer, pH10.3 Magnesium chloride	0.8 mmol/L 10.5 mmol/L
R2	4-nitrophenoxide Magnesium chloride Sodium azide	1000 mmol/L 0.5 mmol/L 1 ml/L

MATERIALS NEEDED BUT NOT PROVIDED

1. Automated chemistry analyzer
2. ALP control and calibrator set (commercially available)

INSTRUMENT

The Kit is applicable on most automated chemistry analyzers. Refer to specific instrument application for suggested settings.

STORAGE AND STABILITY

Store the reagents at 2-8℃. Avoid direct sunlight. The Kit is stable through the expiration date when stored properly. The reagent is stable for 1 month at 2-8℃ after opening.

PRECAUTIONS

1. The Kit is for *in vitro* diagnostic use only. Not for use in humans or animals.
2. The instructions must be followed to obtain accurate results.
3. Do not use the reagents beyond the expiration date.
4. Treat all specimens as infectious. Proper handling and disposal procedures of specimens and test materials should be strictly followed.
5. Reagents contain sodium azide as preservative; avoid contact with skin and eyes, flush with copious amounts of water when disposing.

SPECIMEN COLLECTION AND HANDLING

Follow standard laboratory procedures to collect serum preventing hemolysis. **Do not use EDTA or sodium oxalate-treated plasma samples.**

It is recommended to perform test immediately after sample collection. If the test cannot be done immediately, specimens may be frozen at -20℃ and avoid repeated freeze-thaw cycle.

TEST PROCEDURE (see Figure 1)

Reagent 1 and 2 are liquid stable ready-to-use, no preparation needed.

Calibration: Recommend using commercially available calibrator set for optimal results.

Test procedure: see Figure 1 and Table 1 for instrument parameter setup. Refer to specific instrument application for suggested setting.

1. Add 6 µl of sample and 250 µl of R1; mix well and incubate at 37℃ for 5 minutes.
2. Add 50µl of R2; mix well and incubate at 37℃ for 1 minute.
3. Take continuous optical density measurement for 4 minutes.
4. Calculate average Δ A /min

RESULT

The ALP activity in U/L can be obtained by the following calculation:

$$\text{ALP (U/L)} = (\Delta A_{test}/\text{min} - \Delta A_{blank}/\text{min}) \times \text{factor (F)}$$

The calculation factor for UV spectrophotometer is 2713 when the optical path is 10 mm. Please refer to instrument application if testing under different conditions.

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EXPECTED VALUES

Male: Age 1~12 <500U/L
Age 12~15 <750U/L
Over 25 40~150U/L

Female: Age 1~12 <500U/L
Over 15 40~150U/L

It is recommended for each laboratory to establish its own expected values

QUALITY CONTROL

Using commercially available controls with known concentration is recommended before each batch of tests to ensure the test is properly performed and all reagents and the instrument are functional as specified.

LIMITATIONS

1. The Kit is for *in vitro* use on automated chemistry analyzers only.
2. The test result from the Kit should not be used as the only basis for definite diagnosis.
3. Samples with ALP exceeding the maximum measurement range should be diluted with saline and retested.

PERFORMANCE CHARACTERISTICS

Linearity: 0 - 850 U/L (R≥0.990)

Accuracy: Bias proportion 90%~110%

Precision: Within Run: CV≤5%;
Run-to-Run: CV≤6%

REFERENCES

1. Gaetano Bacci *et al.*, Cancer, 71(4): 1224-1230 (1992).

Not Intended for Sale in the United States.

Pointe Biotech (Nanjing) Co., Ltd.

No.85,Xingmin South Road, Jiang Ning District, Nanjing,P.R.China 211100
Tel:86-25-52425019,
Fax:86-25-52424836
info@biowaydx.com