In Vitro Diagnostics
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This package insert must be read carefully prior to use.

Alkaline phosphatase assay kit for blood tests
(Classification No.: 33165001)

Pureauto S ALP

General Precautions

1. This product is for in vitro diagnostic use, and must not be used for any other purposes.
2. Clinicians should make a comprehensive clinical decision based on assay results in conjunction with clinical symptoms and other examination results.
3. This product should be used only as directed in this package insert. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.
4. If the reagent accidentally comes in contact with eyes and/or mouth, rinse immediately with ample water as first aid, and consult the doctor if required.
5. Carefully read the operating instructions for each type of automated analyzers prior to using this product. Parameters for each type of analyzers are available, and can be requested from SEKISUI MEDICAL CO., LTD. if required.
6. Perform a quality control test prior to assay to ensure accuracy.

Description (Kit Components) **

Component: Ingredients
ALP Buffer Solution 1: Ethylaminoethanol buffer
Magnesium chloride
ALP Substrate Solution 2:
Disodium 4-nitrophenyl phosphate

Intended Use

Measurement of alkaline phosphatase in serum or plasma

ALP is an enzyme that hydrolyzes phosphate compounds at the optimal pH (alkaline). ALP activity is high in organs/tissues such as kidneys (proximal uriniferous tubule), small intestine (mucosa), osteoblasts, placenta, liver, and mammary gland. Blood ALP activity is increased in bone disease, hepatobiliary disease, pregnancy and malignancies.

Assay Principle

1. Assay Principle

4-Nitrophenol (yellow) is released when ALP in samples acts on 4-nitrophenyl phosphate as the substrate in ethylaminoethanol (EAE) buffer. ALP activity is determined by measuring the velocity of 4-nitrophenol production.

4-Nitrophenyl phosphate $\xrightarrow[Mg^{2+}]{ALP}$ 4-Nitrophenol (yellow) + Phosphoric acid

2. Features

1) Final concentration of the principal ingredient matches with the final concentration stipulated in the JSCC Recommendation for Enzyme Activities.
2) Applicable to various automated analyzers.

Procedural Precautions **

1. Properties of Samples and Sampling Methods

1) Samples
Serum and plasma (heparin plasma and citrated plasma) may be used.
2) Storage of samples
The isolated serum (plasma) should be tested on the same day.
If sample cannot be tested on the same day, they should be stored at or below -20°C.[5)]
Bring samples to room temperature (15–30°C) before use.

2. Interfering substances

Assay results are not affected by free bilirubin (up to 40 mg/dL), conjugated bilirubin (up to 40 mg/dL), hemoglobin (up to 500 mg/dL), formazin turbidity (up to 5600 FTU), or ascorbic acid (up to 50 mg/dL).

3. Others

1) Always use Enzyme Calibrator Plus "Daiichi" for calibration.
2) Precautions for assay range
If the activity of sample exceeds assay range, dilute the sample with saline and repeat the measurement.

Dosage/Administration (Assay Procedure) **

1. Preparation of reagents

Reagent (1): ALP Buffer Solution 1 is ready to use.
Reagent (2): ALP Substrate Solution 2 is ready to use.

2. Assay Procedure

This product is compatible with various types of automated analyzer. An example of the assay procedure is indicated below.

Sample 4 μL + Reagent (1) 260 μL $\xrightarrow[5\text{ min}]{37°C}$
Reagent (2) 130 μL $\xrightarrow[96–300\text{ sec}]{37°C}$ Measurement (Absorbance※)
→ Calculation of activity

※ Absorbance: The difference in absorbance between 505 nm and 405 nm
Calibration material: Enzyme Calibrator Plus "Daiichi" (Manufacture's assigned value)
Reagent blank: Purified water or saline

Assessment of Assay Results **

1. Reference standard range[6)]

106–322 U/L

2. There may be reactions or interfering reactions with non-target substances. If assay results appear

to be unreliable, repeat the measurement (if necessary, after dilution) or try another analytical methods.

Performance *

1. **Sensitivity**
   1) Reagent blank: change in absorbance being equal to or lower than 0.002/min
   2) Sensitivity: The change in absorbance is 0.010–0.030/min per 500 U/L of ALP activity.
2. **Accuracy**: 90–110% of the expected assay vale
3. **Within-run Reproducibility**:
   Coefficient of variation ≤5%
   (Test methods used for 1. –3. are in-house methods.)
4. **Measurement Range**[7]: (On Hitachi 7600Ds automated analyzer)
   5–2000 U/L
5. **Correlation** [7]
   1) Serum N=100 r=0.999 y=0.98x –4.7
      Control method: using an already approved in vitro diagnostic product with method in line with the JSCC Recommendation for Enzyme Activities
   2) Plasma N=70 r=0.999 y=0.99x –0.6
      Control method: using an already approved in vitro diagnostic product with method in line with the JSCC Recommendation for Enzyme Activities
6. **Standard Material**
   Enzyme Calibrator Plus, the calibration material used for this product, is in line with the Japanese Standard for Certified Enzyme Reference Materials.

Precautions for Use and Handling **

1. Precautions for Handling (to Ensure Safety)
   1) All samples used in the test should be handled as a material possibly infected with HIV, HBV, HCV, or other viruses. To prevent infection, use disposable gloves and avoid mouth pipetting during the test.
   2) Sodium azide is added as an antiseptic agent in the ALP Substrate Solution 2. Therefore, if the reagent comes in accidentally contact with eyes, mouth or skin, rinse immediately with ample water as first aid, and consult the doctor if required.

2. Precautions for use
   1) This product should be stored as directed, avoid freezing. Freezing can cause deterioration of the reagents, leading to inaccurate results. Therefore, do not use the product if it has been previously frozen.
   2) Do not use expired reagents. Use of such reagents cannot guarantee the reliability of measurement values.
   3) Do not replenish the reagents.
   4) Do not perform the assay under direct sunlight.

3. Precautions for Disposal
   1) Before disposal, used samples and their containers must be immersed in sodium hypochlorite solution at a concentration of greater than 0.1% for longer than 1 hour or autoclaved at 121ºC for 20 minutes.
   2) To prevent infections from spilled samples or solutions containing samples, wipe the spilled area thoroughly with disinfectants such as sodium hypochlorite solution at a concentration of greater than 0.1%.
   3) The reagents and treated samples should be discarded as medical waste or industrial waste according to the waste disposal regulations.
   4) The reagents should be disposed of in accordance with the Water Pollution Control act or related regulations.
   5) Sodium azide is added as an antiseptic agent in the ALP Substrate Solution 2. It can react with lead or copper pipes to produce the highly explosive metal azide. Therefore, the reagent should be flushed with large amounts of water when disposing.

4. Other precautions
   Do not use the containers for other purposes.

Storage and Shelf Life **

1. Storage temperature: 2–10°C
2. Shelf life: 1 year from the date of manufacture
   (The expiration date is printed on the outer package.)

Packaging

Name			Package
Pureauto S ALP	(1)	ALP Buffer Solution 1	4 × 250 mL
	(2)	ALP Substrate Solution 2	4 × 100 mL

Constituent reagents are available in other configurations. For further details please contact SEKISUI MEDICAL CO., LTD.

References **

1) Japan Society of Clinical Chemistry: Jpn J Clin Chem, 19, 209, 1990.
2) Japan Society of Clinical Chemistry: Jpn J Clin Chem , 19, 213, 1990.
3) Osawa S.: Med Tech, 20, 1022, 1992.
4) Japan Society of Clinical Chemistry: Jpn J Clin Chem, 25, 135, 1996.
5) Sasaki M. et al.: Sampling of constituents of the human body, 170, Kodansha, 1972.
6) Kanai M. (supervising editor): Kanai's manual of clinical laboratory medicine. 34th ed. 558, Kanehara Shuppan, 2015.
7) In house data, SEKISUI MEDICAL CO., LTD.

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Manufacturer **

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