

Liquid Reagents – ready to use

Alkaline Phosphatase

opt. DGKC

2 Reagents

Diagnostic reagent for quantitative in vitro determination of alkaline phosphatase (ALP) in human serum or plasma on photometric systems

| Ref.No. | Kit Size | Content |
|----------|-------------|---------------------------------|
| D03102B | 1 x 1.25 L | 1 x 1 L R1 + 1 x 0.25 L R2 |
| D95560 | 5 x 100 mL | 4 x 100 mL R1 + 1 x 100 mL R2 |
| D95561 | 5 x 50 mL | 4 x 50 mL R1 + 1 x 50 mL R2 |
| D00568 | 5 x 25 mL | 4 x 25 mL R1 + 1 x 25 mL R2 |
| D00569 | 5 x 10 mL | 4 x 10 mL R1 + 1 x 10 mL R2 |
| D53911 | 10 x 50 mL | 10 x 40 mL R1 + 4 x 25 mL R2 |
| D0403917 | 5 x 62.5 mL | 4 x 62.5 mL R1 + 1 x 62.5 mL R2 |
| DA0802 | 5 x 50 mL | 5 x 40 mL R1 + 5 x 10 mL R2 |
| DT1002 | 4 x 62.5 mL | 4 x 50 mL R1 + 4 x 12.5 mL R2 |
| DK0702 | 5 x 50 mL | 4 x 50 mL R1 + 1 x 50 mL R2 |
| DE1802 | 2 x 62.5 mL | 2 x 50 mL R1 + 2 x 12.5 mL R2 |

Additionally offered:

| | | | |
|----------|-----------|------------------|-------------|
| D98485 | 5 x 3 mL | Calibrator | Diacal Auto |
| D98485SV | 1 x 3 mL | Calibrator | Diacal Auto |
| D98481 | 12 x 5 mL | Control normal | Diacon N |
| D14481 | 5 x 5 mL | Control normal | Diacon N |
| D98481SV | 1 x 5 mL | Control normal | Diacon N |
| D98482 | 12 x 5 mL | Control abnormal | Diacon P |
| D14482 | 5 x 5 mL | Control abnormal | Diacon P |
| D98481SV | 1 x 5 mL | Control abnormal | Diacon P |

TEST PARAMETERS

| | |
|--------------|--|
| Method: | Colorimetric, kinetic, increasing reaction, optimized DGKC |
| Wavelength: | 405 nm (400 – 420 nm) |
| Temperature: | 37°C |
| Sample: | Serum, heparin plasma |
| Linearity: | up to 800 U/L (manual test procedure) up to 4500 U/L (automatic test procedure) |
| Sensitivity: | The lower limit of detection is 3 U/L |

SUMMARY [1,2]

Alkaline Phosphatase (ALP), a hydrolytic enzyme acting optimally at alkaline pH, exists in blood in numerous distinct forms which originate mainly from bone and liver, but also from other tissues as kidney, placenta, testes, thymus, lung and tumours. Physiological increases are found during bone growth in childhood and in pregnancy, while pathological increases are largely associated with hepatobiliary and bone diseases. In hepatobiliary disease they indicate obstruction of the bile ducts as in cholestasis caused by gall stones, tumours or inflammation. Elevated activities are also observed in infectious hepatitis. In bone diseases elevated ALP activities originate from increased osteoblastic activity as in Paget's disease, osteomalacia (rickets), bone metastases and hyperparathyroidism.

TEST PRINCIPLE



Under alkaline condition, colorless p-nitrophenol is converted to 4-nitrophenoxide, which develops a very intense yellow color. Increase of absorbance is proportional to the activity of alkaline phosphatase in the sample.

REAGENT COMPOSITION

| COMPONENTS | CONCENTRATION |
|------------------------|---------------|
| Reagent 1: | |
| Diethanolamine, pH 9.8 | 1.2 mol/L |
| Magnesium chloride | 0.6 mmol/L |
| Reagent 2: | |
| p-Nitrophenylphosphate | 50 mmol/L |

REAGENT PREPARATION

Substrate Start:

The reagents are ready to use.

Sample Start:

Mix 4 parts of Reagent 1 with 1 part of Reagent 2 (= Working Reagent)

REAGENT STABILITY AND STORAGE

| | |
|-------------|--|
| Conditions: | Protect from light. Close immediately after use. Avoid contamination. Do not freeze the reagents! |
|-------------|--|

Substrate Start:

Storage: at 2 – 8 °C

Stability: up to the expiration date indicated on labels

Sample Start (Working Reagent):

| | | |
|------------|---------------|---------|
| Stability: | at 2 – 8 °C | 4 weeks |
| | at 15 – 25 °C | 5 days |

The working reagent must be protected from light!

SAMPLE STABILITY AND STORAGE

| | | |
|----------------|---------------|----------|
| Stability [4]: | at 20 – 25 °C | 7 days |
| | at 4 – 8 °C | 7 days |
| | at - 20 °C | 2 months |

Discard contaminated specimens!

Freeze only once!

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)

General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Substrate Start

| | |
|---|---------|
| Pipette into test tubes | 37°C |
| Reagent 1 | 1000 µL |
| Sample | 20 µL |
| Mix. Incubate for approximately 1 minute. Then add: | |
| Reagent 2 | 250 µL |
| Mix. Read initial absorbance against air after 1 minute and start a stopwatch. Read absorbance again after exactly 1, 2 and 3 min. Determine ΔA/min. during the linear part of the assay. | |

Sample Start

| | |
|---|---------|
| Pipette into test tubes | 37°C |
| Working Reagent | 1000 µL |
| Sample | 20 µL |
| Mix. Read initial absorbance against air after 1 minute and start a stopwatch. Read absorbance again after exactly 1, 2 and 3 min. Determine ΔA/min. during the linear part of the assay. | |

CALCULATION

With factor: (light path 1 cm)

Alkaline Phosphatase [U/L] = ΔA/min x Factor

| | | |
|--------------------------------|------------------|------|
| Factors (405 nm, 37°C): | Substrate Start: | 3433 |
| | Sample Start: | 2757 |

With calibrator:

$$\text{ALP [U/L]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}} \times \text{Conc. Calibrator [U/L]}$$

UNIT CONVERSION

U/L x 0,01667 = µkat/L

REFERENCE RANGES [6] * (37 °C)

| | Year(s) | U/L | µkat/L |
|------------------|--------------|-------|--------|
| Adults: | | < 258 | < 4.30 |
| Children: | 1–12 year(s) | < 727 | < 12.1 |
| Females | 13-17 years | < 448 | < 7.47 |
| Males | 13-17 years | < 935 | < 15.6 |

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

On automated systems the test is suitable to determine alkaline phosphatase activities up to 4500 U/L. In case of a manual procedure, the test is suitable for alkaline phosphatase activities up to 800 U/L which correspond to a maximum $\Delta A/\text{min}$ of 0.25. If the value is exceeded, the sample should be diluted 1 + 9 with NaCl solution (9 g/L) and the result multiplied by 10.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 3 U/L

PRECISION

| Intra-assay n = 20 | Mean [U/L] | SD [U/L] | CV [%] |
|-----------------------|---------------|-------------|-----------|
| Sample 1 | 114 | 1.71 | 1.50 |
| Sample 2 | 222 | 2.05 | 0.92 |
| Sample 3 | 275 | 2.91 | 1.06 |

| Inter-assay n = 20 | Mean [U/L] | SD [U/L] | CV [%] |
|-----------------------|---------------|-------------|-----------|
| Sample 1 | 120 | 1.93 | 1.60 |
| Sample 2 | 223 | 1.89 | 0.85 |
| Sample 3 | 279 | 2.36 | 0.85 |

SPECIFICITY/INTERFERENCES

no interference up to:

| | |
|---------------|------------|
| Ascorbic acid | 30 mg/dL |
| Bilirubin | 40 mg/dL |
| Hemoglobin | 150 mg/dL |
| Triglycerides | 2000 mg/dL |

For further information on interfering substances refer to Young DS [5].

METHOD COMPARISON

A comparison of Dialab Alkaline phosphatase opt. DGKC (y) with a commercially available test (x) using 78 samples gave following results:
 $y = 0.98x - 2.21$ U/L; $r = 0.999$.

CALIBRATION

The use of an Alkaline Phosphatase Calibrator is optional. We recommend the Dialab multi calibration serum **Diacal Auto**. This method is traceable to the molar extinction coefficient.

QUALITY CONTROL

All control sera with Alkaline Phosphatase values determined by this method can be used.

We recommend the Dialab serum controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range).

Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION

Special applications for automated analysers can be made on request.

WARNINGS AND PRECAUTIONS

- Reagent 1: Danger.
 H315: Causes skin irritation.
 H318: Causes serious eye damage.
 H373: May cause damage to organs through prolonged or repeated exposure.
 P260: Do not breathe vapors.
 P280: Wear protective gloves/protective clothing/eye protection/face protection.
 P302+P352: If on skin: Wash with plenty of water/soap
 P305+P351+P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
 P310: Immediately call a poison centre/doctor.
- Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
- During reaction p-nitrophenol is produced which is poisonous when inhaled, swallowed or absorbed through skin. If the reaction mixture comes in contact with skin or mucous membranes wash copiously with water!

- In very rare cases, samples of patients with gammopathy might give falsified results [7].
- Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
- For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
- For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements

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