# ALKALINE PHOSPHATASE LR liquid reagent

**REF C3000620/C3000620A** 6x20ml
**C3000650/C3000650A** 6x50ml

CE IVD For in vitro medical device

## Use

Kit for measurement of alkaline phosphatase in serum or plasma.
Colorimetric optimized method DEA DGKC.

## Summary

Alkaline phosphatase measurements are used in the diagnosis and treatment of hepatobiliary and bone disease.

## Principle

Kinetic analysis. Alkaline phosphatase (ALP) catalyzes the hydrolysis, in alkaline environment, of p-nitrophenylphosphate into p-nitrophenol and phosphate. The rate of increase in absorbance due to the formation of p-nitrophenol is directly proportional to sample ALP activity.

## Reagents

| | | |
|---|---|---|
| **R1** | Diethanolamine buffer | pH 9.8 3.5 mol/l |
| | magnesium chloride | 1.0 mmol/l |
| **R2** | P-nitrophenylphosphate | 45.0 mmol/l |

## Reagents Preparation

Reagents are liquid and ready to use. About use as monoreagent ("sample-starter" procedure) add the entire content of one bottle of Alkaline phosphatase **R2** in the alkaline phosphatase **R1** bottle and mix gently. For minor use add for every 4 ml of R1 reagent, 1 ml of R2 reagent. Keep out the reagents from refrigerator only for the use and recap them immediately.

## Storage and stability

- Store the kit at 2-8°C.
- After opening, the vials R1 and R2 are stable 90 days if recapped immediately and protected from contamination, evaporation, direct light, and stored at the correct temperature.
- Working solution stability (R1+ R2): 20 days at 2-8°C.

## Precaution

The product is not classified as dangerous (DLg. N. 285 art. 28 l. n. 128/1998).
However the reagent should be handled with care, according to good laboratory practice.
Caution: the reagents contain Sodium Azide (0.095%) as preservative. Avoid swallowing and contact with skin, eyes and mucous membranes.
The reagent R1 contains diethanolamine; risk of serious damage for eyes; dangerous for ingestion (**R41- R48/22).**

Waste Management

Please refer to the local legal requirements.

## Specimen Collection and Preparation

- Serum or heparinized plasma.
- Do not use samples with haemolysis. The withdrawal must be made, preferably, after 8 hours of fasting. The anticoagulants as EDTA, citrate and oxalate must not be used because they inhibit the enzyme activity.
- The ALP is stable in the samples up to 7 days at 2-8°C.

## Note

- The kit, according to this method, must be used in manual procedures. About automatic use follow specific applications.
- Avoid direct light, contamination and evaporation.
- The volumes in the procedure can be changed proportionally.
- In case of complaint or quality control request, refer to the lot number on the package or the lot number on the singles vials.

## Procedures

| | |
|---|---|
| Wavelength | λ: 405 (400- 410) nm |
| Working temperature | 37°C |
| Optical path | 1 cm |
| Reaction | "kinetic" (increasing) |

Bring the reagents at 15-25°C before use.

### Monoreagent Procedure "sample starter"

| | BLANK | STD | SAMPLE |
|---|---|---|---|
| **Working reagent** | 1000 µl | 1000 µl | 1000 µl |
| **Distilled Water** | 20 µl | -- | -- |
| **Sample** | -- | -- | 20 µl |
| **Standard** | -- | 20 µl | -- |

Mix, then incubate for 1' at 37°C. Measure the absorbance of sample (EC) against distilled water decrease per minute during 3 minutes. Calculate the absorbance variation ΔE/min from performed readings.

### Bireagent Procedure "substrate starter"

| | BLANK | STD | SAMPLE |
|---|---|---|---|
| **Reagent R1** | 800 µl | 800 µl | 800 µl |
| **Distilled Water** | 20 µl | -- | -- |
| **Sample** | -- | -- | 20 µl |
| **Standard** | -- | 20 µl | -- |

Mix, incubate at 37°C for 1' and then add:

| | BLANK | STD | SAMPLE |
|---|---|---|---|
| **Reagent R2** | 200 µl | 200 µl | 200 µl |

Mix, then incubate for 1' at 37°C. Measure the absorbance of sample (EC) against distilled water decrease per minute during 3 minutes. Calculate the absorbance variation ΔE/min from performed readings.

## Calculation

ALP [U/l] = ΔE/min x 2757

The factor and the reagent performances are related to 37°C, 1 cm and 405 nm.

## Reference Values to 37°C

Adults 100 – 290 (U/l)
Children 180 – 1200 (U/l)

Reference values are considered indicative since each laboratory should establish reference ranges for its own patient population. The analytical results should be evaluated with other information coming from patient's clinical history.

## ANALYTICAL PERFORMANCES

### Linearity

Reaction is linear up to a concentration of 1600 U/l. Samples with values exceeding this range must be diluted with saline solution. Then multiply the result for diluting factor.

### Analytical Sensitivity

The test sensitivity in terms of detection limit is 9.80 U/l.

### "Intra-Assay" precision (within-Run)

Determined on 20 samples for each control (N-H) (Normal-High). Results:

| | | |
|---|---|---|
| MEAN (U/l) | N = 179.35 | H = 460.95 |
| S.D. | N = 1.56 | H = 3.07 |
| C.V.% | N = 0.87 | H = 0.67 |

### "Inter-Assay" precision (between-run)

Determined on 20 samples for each control (N-H). Results:

| | | |
|---|---|---|
| MEAN (U/l) | N = 173.23 | H = 461.50 |
| S.D. | N = 2.17 | H = 3.36 |
| C.V.% | N = 1.27 | H = 0.73 |

### Correlation

A study based comparing this method with a similar method on 20 samples has given a correlating factor **r = 0.99**

**y = 1.016 x + 8.299**

### Interferences

No interferences was observed by the presence of :

| | |
|---|---|
| Bilirubin | ≤ 25 mg/dl |
| Triglycerides | ≤ 800 mg/dl |
| Ascorbate acid | ≤ 20 mg/dl |
| Haemoglobin | ≤ 100 mg/dl |

For a comprehensive review of interfering substances, refer to the publication by Young.

### Quality controls

It's necessary, each time the kit is used, to perform quality controls and to check that values obtained are within the acceptance range provided in the insert. Each laboratory should establish its own mean and standard deviation and adopt a quality control program to monitor laboratory testing.

### Bibliography


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Kubler W: Symp. D. Deutsch Ges. fur Lab. Med., 8 (1973). Fischbach F, Zawta B: Klin. Lab., 38, 555 (1992). Szasz G. et al.: Z. Kindrheilk, 111, 233 (1971). Kaplan LA, Pesce AJ: "Clinical Chemistry", Mosby Ed. (1996).
Young D.S., Effects of Drugs on Clinical Laboratory Tests, AACC Press, Washington, DC 5th ed.2000.


### Symbols

| | |
|---|---|
| CE | CE Mark (98/79 CE regulation) |
| IVD | in vitro medical device |
| LOT | Batch Code |
| | Use by |
| | Storage temperature limits |
| | Read instruction for use |
| | Producer |




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