

ALDOLASE

KINETIC UV DETERMINATION IN SERUM
 Only for in vitro diagnostic use

Kit: 1x100 mL

Cod. ALD100

SUMMARY

The Aldolase is a very important lyase who manage the splitting of Fructose-1,6-diphosphate to glyceraldehyde-P and dihydroxyacetone-P, a very important reaction in the glycolytic breakdown of glucose to lactate. The greatest clinical interest is in the primary diseases of skeletal muscle.

PRINCIPLE

This Reagent determines the aldolase activity in vitro; in the presence of the substrate D-Fructose-1,6-bisphosphate and of the enzymes who manage the ancillary reactions, TIM (triosephosphate isomerase)-GDH(glycerol-3P-dehydrogenase)-LDH(lactate dehydrogenase), at the end the aldolase change NADH to NAD.

The decrease of absorbance of NADH, for oxidation to NAD, is proportional to the activity of the Aldolase in the sample.

REAGENTS

Components of the kit:		Code ALD100
*REAGENT 1 (liquid)	to dilute to	1 x 100 mL
*REAGENT 2 (lyo)	to dilute to	1 x 2 mL
*REAGENT 3 (susp.)		1 x 0,7 mL
Collidina buffer	56 mmol/L pH 7,4	
F-1,6-PP	3 mmol/L	
NADH	0,22 mmol/L	
GDH	≥ 300 U/L	
TIM	≥ 4000 U/L	
LDH	≥ 500 U/L	

STABILITY: the reagents, at 2-8°C, are stable up to the expiry date shown on the package if **not contaminated during handling**.

AUXILIARY REAGENTS (Not supplied with this kit)

To assure proper test performance, we suggest following kits:

- LEVEL 1 ENZYME Control 5 x 3 mL Cod. BEE1005
- LEVEL 2 ENZYME Control 5 x 3 mL Cod. BEN2005

that contains a few enzymes in different ranges (see the inserts).

PREPARATION OF THE WORKING REAGENT

*REAGENT 1 (liquid).

Dilute the content of the bottle to 100 mL with distilled water; mix gently until dissolution.

STABILITY: 4 weeks at 2-8°C, in the dark.

*REAGENT 2 (lyo).

Dilute the content of the bottle to 2 mL with distilled water; mix gently until dissolution.

STABILITY: 4 weeks at 2-8°C, in the dark.

*REAGENT 3 (susp.).

Ready to use.

Mix kindly and let the working reagent reaches the working temperature before use.

Close immediately after handling.

Incompetent handling will release us from any responsibility.

SAMPLE

- No haemolyzed fresh serum or plasma (heparin or EDTA).

PROCEDURE

- Wavelength: 340 nm (334-365 nm)
- Pathlength: 1 cm
- Reading: against air or Distil. water
- Temperature: 37°C
- Method: kinetic
- Reaction: 20 minutes
- Linearity: till 27 U/L
- Sample/Reagent: 1/12,5

Let reagents reach the working temperature before using.

Pipette into a test tube or cuvette labelled:

R/B: SAMPLE/Calibrator Blank; S: Sample; ST: Calibrator/Standard :

	R/B	ST and/or S	
*Reagent 1	----	2500 µl	
Saline Sol. (9 g/L NaCl)	2500 µl	----	
*Reagent 2	----	50 µl	
*Reagent 3	----	10 µl	
Sample/Calibrator	200 µl	200 µl	

Mix carefully and incubate for 5 min. at 37°C.

Measure the initial absorbances for each one under test (Calibrator and/or Samples) against the SAMPLE/Calibrator Blank (R/B); call **A1** for each one.

Let the samples or Calibrator for 20 min. at 37°C.

Measure the FINAL absorbances for each one under test (Calibrator and/or Sample) against the SAMPLE/Calibrator Blank (R/B); call **A2** for each one.

ΔA = A1 - A2 (for each sample or Calibrator under test)

CALCULATION

$\Delta A \times 54,8 =$ ALDOLASE activity in U/L at 340 nm.

REFERENCE VALUES

Serum or plasma: till 7,6 U/L at 340 nm and at 37°C.

It is advisable that every laboratory determine its normal reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

Linearity: Aldolase may be determined between 0,1 - 27 U/L. For concentrations ≥ 27 U/L, dilute the sample 1:10 with saline sol., repeat the determination and multiply the result $\times 10$.

Sensitivity: The minimum detectable is 0,1 U/L.

Within-run Precision:

	Mean (U/L) $\pm 2s$	CV %
Serum 1	8,2 \pm 0,28	1,72
Serum 2	19,8 \pm 0,4	1,11

Run-to-run (Day-to-day) Precision:

	Mean (U/L) $\pm 2s$	CV %
Serum 1	8,3 \pm 0,38	2,27
Serum 2	20,2 \pm 0,93	2,30

Interferences: See References point 2.

Correlation: A group of 20 sera from 1 to 23 U/L was assayed by this procedure and using a similar commercially available Aldolase Reagent. Comparison of the data gave following results:

Linear regression equation $y = 1,0232x - 0,24$
 Correlation coefficient $r = 0,9993$

NOTE

1. A proportional variation of the reaction volumes does not change the result.
2. We suggest do not mix Reagents from different Production lots.
3. Dilute the sample 1:10 with saline solution if activity is higher than 27 U/L (37°C), repeat the test and multiply the result x 10.
4. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of Aldolase (see References 2.).

5. PAY ATTENTION!

Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.

6. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
7. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975).
3. Beisenherz, G., et al., Z. Naturforsch., 8(b), 555 (1953).

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