LYSOZYME

Turbidimetric Determination in Urina, Serum and Plasma

REF CY07-72 12 x 6 ml **REF CY07-150** 10 x 15 ml

Additional required reagent:

1 x 1 ml LYSOZYME STANDARD

REF 7500

Available for quality control:

LYSOZYME CONTROL L1+L2

REF 7522

Control Urine in normal and pathological range

PRINCIPLE

Lysozyme catalyzes hydrolysis of β -glucosidic bonds of polysaccharides in the cell membrane. The turbidity decrease of a suspension of Micrococcus lysodeikticus cell membranes is proportional to the enzymatic activity and is related to lysozyme concentration by a calibration curve.

REAGENT

Kit composition:	REF CY07-72	Quantity	REF CY07-72	Quantity
REAGENT 1 (lyo)	CY07-72R1	12 vials	CY07-72R1	10 vials
M. lysodeikticus				

STABILITY: store at 2-8°C to keep the reagents stable up to the expiration date on the label

PREPARATION OF WORKING REAGENT

REF CY07-72

Reconstitute a vial of Reagent 1 with exactly 6.2 ml of distilled water. Shake gently to obtain an uniform suspension.

REF CY07-150

Reconstitute a vial of Reagent 1 with exactly 15.5 ml of distilled water. Shake gently to obtain an uniform suspension.

STABILITY: 48 hours at 2-8°C.

SAMPLE

STABILITY: urine with pH ranged between 4,5 and 6,3 is stable 4 days at room temperature, 1 week at 2-8°C, 2 weeks at -20°C.

The stability decreases with pH higher values.

Serum and plasma.

Do not use heparin as anticoagulant.

STABILITY: 2 weeks at -20°C

MANUAL ASSAY PROCEDURE

Wavelength: 570 nm (450, 540 or 640 nm)

Optical path: 1 cm

Reading: against air or distilled water

Temperature: Reaction time: 2 minutes Linearity: up to 50 mg/L Reagent/sample:

Let the working reagent reach 25°C before using it in the test.

Pipette into a cuvette:

Working reagent	480 μl	
Sample	8 µl	

Mix accurately. Incubate for 30 seconds. Read the absorbance (A1). After exactly 2

minutes, read the absorbance (A2)

Calculate the absorbance difference ΔA (2 min) = A1 - A2.

CALCULATION

As further on described, draw a calibration curve using LYSOZYME STANDARD

Calculate the concentration in the samples by the calibration curve.

CALIBRATION CURVE

To draw the calibration curve, dilute the LYSOZYME STANDARD 500 mg/L with saline solution, to obtain working solutions with lysozyme concentrations ranged between 2 and 20 mg/L.

For example, proceed as it follows:

FIRST DILUTION

Dilute 0.4 ml of LYSOZYME STANDARD 500 mg/L with to 3.6 ml of saline solution. Thus 50 mg/L of LYSOZYME STANDARD are obtained.

Mix well the obtained solution.

SECOND DILUTION

Proceed as described in the following table, mixing well the solutions before use:

LYSOZYME STANDARD 50 mg/L	+	Saline solution	=	Lysozyme final concentration
0,4 ml	+	0,6 ml	=	20 mg/L
0,2 ml	+	0,8 ml	=	10 mg/L
0,1 ml	+	0,9 ml	=	5 mg/L
0,1 ml	+	2,4 ml	=	2 mg/L

REFERENCE VALUES

Urine: fino a 2 mg/L 2.5 - 8.0 mg/L Serum or plasma:

PERFORMANCE CHARACTERISTICS

Linearity: up to 50 mg/L.

For higher values, properly dilute the samples and multiply the result by the dilution factor (see also note 3).

Within run precision:

	Level 1	Level 2
Average (mg/L)	1.01	15.0
DS	0.0085	0.207
CV %	0.84	1.38

Between run precision:

Level 1	Level 2
1.45	16.5
0.0205	0.428
1.41	2.59
	1.45 0.0205

Correlation: FAR kit for lysozyme determination shows a correlation coefficient of 0.988 compared to another kit commercially available.

NOTES

- Read the information in the MSDS.
- As Micrococcus lysodeikticus cell sensibility to lysozyme action varies in the 2. different lots, draw the calibration curve with same lot of reagent used in the
- For values of ΔA (2 min) higher than 0,1 spectrophometric unit, dilute 1 volume of the sample with 4 volumes of saline solution.
- Repeat the assay and multiply the result by 5. Each laboratory should define its own reference values.
- 4. 5. Reaction volumes can be proportionally changed.
- 6. Measures can be performed in ELISA plates.
- 7. Disposal according to local laws.
- 8. Chemistry analyzer parameters are available.

REFERENCES

- Rudders et Bloch. Am.J.medical sciences (1971: 79-85)
- Horpcsy et al. Clin. Chem. 24/1 74-79. 1978)

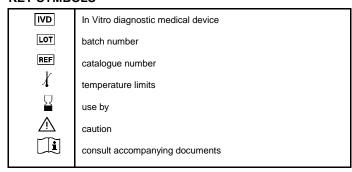
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KEY SYMBOLS



Issue 01 - Jan 2021 RR