LYSOZYME

Turbidimetric Determination
in Urina, Serum and Plasma

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

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) *M. lysodeikticus*	**CY07-72R1**	**12 vials**	**CY07-72R1**	**10 vials**

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

Urine.
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:	25°C
Reaction time:	2 minutes
Linearity:	up to 50 mg/L
Reagent/sample:	60/1

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 (REF 7500) kit.
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
Serum or plasma:	2.5 - 8.0 mg/L

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
Average (mg/L)	1.45	16.5
DS	0.0205	0.428
CV %	1.41	2.59

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

NOTES

1. Read the information in the MSDS.
2. As *Micrococcus lysodeikticus* cell sensibility to lysozyme action varies in the different lots, draw the calibration curve with same lot of reagent used in the assay.
3. 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.
4. Each laboratory should define its own reference values.
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

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

MANUFACTURER

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalogue number
	temperature limits
	use by
	caution
	consult accompanying documents

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