LysozymeMicroplate Assay Kit

User Manual

Catalog # CAK1282

(Version 1.1A)

Detection and Quantification of LysozymeActivity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Lysozyme catalyzes the hydrolysis of §-1,4 glucosidic linkages which occur in the cell walls of microorganisms. It is widely distributed in animals and plants. Lysozyme from chicken egg white has been extensively studied. It is a basic protein with a molecular weight of approximately 14,000.

Lysozyme is normally present in plasma (5.9 mg/L) but only in trace amounts in urine. In certain renal disorders, urinary excretion of lysozyme is significantly increased, which could be of diagnostic significance. Analysis of serum lysozyme levels could also be used as a diagnostic tool in acute and chronic myelocytic leukemia and in acute lymphocytic leukemia.

Lysozyme Microplate Assay Kit provides ready-to-use reagents for detecting the presence of lysozyme activity. This simple assay to detect lysozyme activity uses Micrococcus lysodeikticus cells as the substrate.Lysozyme activity results in the lysis of the Micrococcus lysodeikticus cells. During incubation of the lysozymesample and substrate, the reaction is followed by monitoring the decrease in absorbance at 450 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
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Note:

Substrate: add 3 mlReaction Buffer to dissolve before use, it is a suspension.

Standard: add 0.5 ml Assay Bufferto dissolve before use, the concentration will be 20000 U/ml.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay bufferfor 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Warmall reagentsto25°C before use, and the substrate need to be mixed before adding to the plate.

Add following reagents intothe microplate:

Reagent	Sample	Blank	Standard
Reaction Buffer	160 µl	160 µl	160 µl
Substrate	30 µl	30 µl	30 µl
Sample	10 µl	--	--
Assay Buffer	--	10 µl	--
Standard	--	--	10 µl
Mix, incubate at room temperature for 5 minutes,recordabsorbance measured at 450 nm.			

Note:

1) If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

2) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{Lysozyme (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Blank} - OD_{Sample}) / (OD_{Blank} - OD_{Standard}) / (V_{Sample} \times C_{Protein})$$

$$= 20000 \times (OD_{Blank} - OD_{Sample}) / (OD_{Blank} - OD_{Standard}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Lysozyme (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Blank} - OD_{Sample}) / (OD_{Blank} - OD_{Standard}) / (V_{Sample} \times W / V_{Assay})$$

$$= 20000 \times (OD_{Blank} - OD_{Sample}) / (OD_{Blank} - OD_{Standard}) / W$$

3. According to the quantity of cells or bacteria

$$\text{Lysozyme (U/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Blank} - OD_{Sample}) / (OD_{Blank} - OD_{Standard}) / (V_{Sample} \times N / V_{Assay})$$

$$= 20000 \times (OD_{Blank} - OD_{Sample}) / (OD_{Blank} - OD_{Standard}) / N$$

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the standard activity, 20000 U/ml;

W: theweight of sample, g;

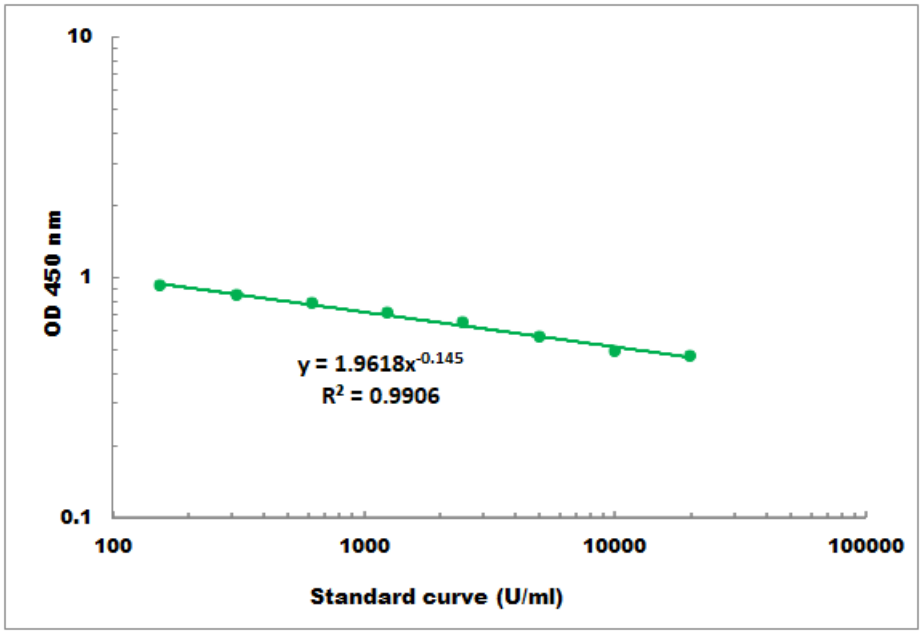
$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

N: the quantity of cell or bacteria, N $\times 10^4$.

VII. TYPICAL DATA



Detection Range:100 U/ml - 20000 U/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES