(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Lysozyme (LZM) Assay Kit

Catalog No.: BC00214

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Lysozyme (LZM) Assay Kit
Detection Method	Turbidimetry (Measure the light transmittance)
Sample Type	Serum, plasma, tissue, urine, saliva
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (530 nm)

Different samples correspond to different determination methods

Product Introduction

Lysozyme, also known as muramidase or N-acetylmuramide glycanohydrolase, is an alkaline enzyme that hydrolyzes mucopolysaccharides in pathogenic bacteria. It primarily breaks the β-1,4 glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine in bacterial cell walls. This decomposes the insoluble mucopolysaccharides of cell walls into soluble glycopeptides, causing cell wall rupture and leakage of intracellular contents, which ultimately leads to bacterial lysis.

Additionally, lysozyme can directly bind to negatively charged viral proteins and form complexes with DNA, RNA and apoproteins, thereby inactivating viruses. Therefore, this enzyme exerts antibacterial, anti-inflammatory and antiviral effects.

Principle

In a turbid bacterial suspension of fixed concentration, lysozyme hydrolyzes peptidoglycan in bacterial cell walls and causes bacterial lysis. This reduces turbidity and increases light transmittance. Accordingly, the content of lysozyme can be determined based on changes in light transmittance.

Components

No.	Components	Size	Notes
Reagent 1	Bacterial powder	5 mg×4	Store in a dry place at 2-8°C.
Reagent 2	Bacterial powder diluent	100 mL	Store in a dry place at 2-8°C. Sterilize in a microwave oven for 20 seconds every month.
Reagent 3	Standard powder	2 mg	Store in a dry place at 2-8°C.

Storage

The kit can be stored for 6 months under specified conditions.

Preparation

Spectrophotometer (with 530 nm wavelength), 1 cm path length cuvettes, 37 °C water bath, bench-top low-speed centrifuge, pipettes of various specifications, glass homogenizer (for grinding bacterial powder, available from our company), double distilled water, 0.9% normal saline or 0.1M PBS, vortex mixer, test tubes or centrifuge tubes, protein assay reagents (for tissue samples, available from our company).

- **Reagent preparation**

1. Preparation of Stock Bacterial Suspension: Take one vial of bacterial powder (5 mg per vial), add 1 mL of bacterial powder solvent and mix well. Transfer the mixture to a homogenization tube, then grind gently and slowly with up-and-down rotation for 3 minutes (avoid splashing). The resulting solution is the stock bacterial suspension. Seal it tightly and store in a refrigerator at 2-8 °C; it can be preserved for approximately one week.
2. Preparation of Working Bacterial Suspension: Prepare at the ratio of stock bacterial suspension to bacterial powder diluent = 1:19. Prepare the required volume as needed and shake well before use.
3. Preparation of Standard Stock Solution: Accurately add 1.0 mL of double distilled water to each

vial to prepare the standard stock solution with a concentration of 2 mg/mL. Store at 2-8 °C for 7 to 10 days.

4. Preparation of Working Standard Solution: Prior to use, dilute the standard stock solution with double distilled water at a ratio of 1:99 to prepare a working standard solution of 20 μg/mL. Further dilute this 20 μg/mL standard solution 8-fold (1:7) with double distilled water to obtain a working standard solution of 2.5 μg/mL (equivalent to 200 U/mL). Prepare the solution in required volume only. Store at 2-8 °C for 7 to 10 days.

Operation process

- **Determination of Lysozyme by Self-control Method**

Suitable for all samples

1. Place the prepared working bacterial suspension in a 37°C water bath and preheat for no less than 10 minutes to reach 37°C. Ensure the standard solution and samples are maintained at the same temperature.
2. Set the visible spectrophotometer to 530 nm with 1 cm path length cuvettes. Use double distilled water to adjust the transmittance to 100%. Prepare two cuvettes: one for blank calibration and the other for sample measurement.
3. Add 0.2 mL of the test sample into the correspondingly numbered test tube. Quickly pour 2 mL of working bacterial suspension into the tube, mix thoroughly and start timing immediately. For the working standard solution, take 0.2 mL and add 2 mL of working bacterial suspension; perform all subsequent operations identically.
4. Immediately transfer the mixture into the cuvette. Record the transmittance value (T_0) at 15 seconds under 530 nm. Keep the cuvette in place and record the transmittance value (T_2) at 2 minutes and 15 seconds.
5. Calculate the difference between the two transmittance readings: $\Delta T = T_2 - T_0$.

Notes:

1. Mix the working bacterial suspension periodically during use.
2. If the ΔT of the test tube is greater than 10, dilute the sample and re-test.
3. Rinse the cuvette thoroughly with double distilled water after each sample or working standard solution before the next measurement.

Calculation

$$\text{Lysozyme content in liquid samples (μg/mL)} = \frac{\Delta T_{Assay}}{\Delta T_{Standard}} \times C_{Standard} \times N$$

$$\text{Lysozyme content in tissue samples (μg/mg)} = \frac{\Delta T_{Assay}}{\Delta T_{Standard}} \times C_{Standard} \div Cpr$$

Annotation:

$C_{Standard}$: Standard solution concentration, 2.5μg/mL

N: Dilution factor of the sample before testing

Cpr: Tissue homogenate protein concentration, mg/mL

- **Determination of Lysozyme by Blank Control Method**

  Applicable to colorless and clear samples, such as partial serum, urine and saliva.

  The following operations are preferably performed in an ice water bath.

	Blank well	Standard well	Assay well
Double distilled water (mL)	0.2		
2.5 μg/mL Working Standard Solution (mL)		0.2	
Sample (mL)			0.2
Working Bacterial Suspension (mL)	2.0	2.0	2.0
Mix well, then incubate accurately in a 37°C water bath for 15 minutes. Immediately transfer to an ice water bath below 0°C for 3 minutes. Transfer the solution of each tube into 1 cm path length cuvettes one by one. Adjust the transmittance to 100% with double distilled water at 530 nm, then measure the transmittance T.			

Notes:

1. Mix the working bacterial suspension periodically during use.
2. Fog may form on the cuvette surface during reading. Wipe the cuvette thoroughly before taking

readings.

3. To eliminate cross-interference between test tubes during measurement, rinse the cuvette with double distilled water before testing each tube.
4. If the difference between the transmittance of the test tube and the blank tube exceeds 15, dilute the sample and re-measure.

Calculation

$$\text{Lysozyme content } (\mu g/mL) = \frac{T_{Assay} - T_{Blank}}{T_{Standard} - T_{Blank}} \times C_{Standard} \times N$$

Annotation:

$C_{Standard}$: Standard solution concentration, 2.5μg/mL

N: Dilution factor of the sample before testing

- **Determination of Lysozyme by Standard Curve Method**

  Applicable to colorless and clear samples, such as partial serum, urine and saliva.

1. Dilution of Standard Solution: Accurately add 1.0 mL of double distilled water to one vial of standard substance and mix well to prepare the standard stock solution at a concentration of 160000 U/mL. Subsequently, further dilute it with double distilled water to obtain standard solutions with serial concentrations: 2000 U/mL, 1000 U/mL, 500 U/mL, 250 U/mL, 125 U/mL, 62.5 U/mL, 31.25 U/mL and 15.625 U/mL.
2. Place lysozyme standard solutions of various concentrations at the same temperature as the samples for no less than 5 minutes. Preheat the working bacterial suspension at 37 °C for more than 10 minutes.
3. Follow the procedures in the table below (preferably perform the operations in an ice water bath).

	Blank well	**Standard well**
Double distilled water (mL)	0.2	
Standard solutions of different concentrations (mL)		0.2
Working Bacterial Suspension (mL)	2.0	2.0

Incubate accurately in a 37°C water bath for 15 minutes, then immediately transfer to an ice water bath below 0°C for 3 minutes. Transfer the solution from each tube into 1 cm path length cuvettes sequentially. Set the spectrophotometer to 530 nm and adjust the transmittance to 100% using double distilled water, then measure the transmittance T.

4. Plotting: Take the unit concentration of each standard tube as the abscissa and the transmittance difference of each tube as the ordinate to draw the standard curve.
5. Determine the samples in accordance with the operating table for lysozyme assay by blank control method, then calculate the lysozyme content in the samples based on the standard curve.

Precautions

1. The values measured by spectrophotometer in this method must be transmittance readings.
2. It is recommended to use disposable laboratory test tubes for operation.
3. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
4. Grind the bacterial powder thoroughly until fine and homogeneous, and avoid splashing during grinding.
5. Pre-cool the bacterial suspension, standard solutions and samples in an ice water bath at 0 °C for no less than 5 minutes. All operations prior to 37°C water bath incubation is preferably carried out in an ice water bath.
6. To minimize detection errors, use a dedicated cuvette for the blank tube. Rinse cuvettes with tap water first, followed by 2 to 3 washes with double distilled water before each sampling and measurement.
7. Take tubes out of the ice water one by one for absorbance measurement. Wipe off water mist on cold cuvette surfaces completely before testing.
8. Use test tubes from the same batch.
9. Calibrate all 1 cm path length cuvettes to reduce experimental errors.
10. Synchronize spectrophotometry and timing strictly to ensure accurate time control. The assay is recommended to be performed by two operators or using an automatic biochemical analyzer.
11. In the self-control method, if the ΔT value of the sample is less than 0.5, extend the reaction time. Meanwhile, dilute the standard solution by the same multiple as the time extension.