Ver.260201

Lysozyme (LZM) Assay Kit

BC091(30T/28S)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

1. Product Principle

In a turbid bacteria-containing liquid, lysozyme catalyzes the hydrolysis of peptidoglycan in the bacterial cell wall, causing bacterial lysis. This reaction increases the transmissivity of the liquid. Therefore, the lysozyme (LZM) content can be determined by measuring the change in transmissivity.

2. Reagent composition

Reagents	Volume	Storage
Bacteria powder	5mg × 4 vials	2 -8℃
Keep away from moisture for 6 months		
Bacteria powder diluent	100mL	2 -8℃
Please use microwave oven to autoclave for 20 seconds each month		
Standard	2mg × Powder	2 -8℃
Keep away from moisture for 6 months		

NOTE: If the reagent is unopened and stored strictly according to the above method, its shelf life can be extended to 1 year.

Reagent preparation:

I. **Bacteria stock solution preparation**:

Take one vial of bacterial powder (5mg/vial), add 1mL of bacterial powder solvent, mix well, then transfer to a homogenization tube. Gently and slowly grind by rotating up and down for about 3 minutes (do not splash out). This is the stock bacterial suspension. After preparation, store sealed at 2–8℃; it can be stored for about one week.

II. **Preparation of working bacterial suspension:**

Prepare according to **stock bacterial suspension: bacterial powder solvent = 1:19**. Prepare only the amount needed and mix well before use.

III. **Preparation of standards:**

Preparation of standard stock solution:

Accurately add 1.0mL of double-distilled water to each vial to prepare a 2mg/mL standard stock solution. It can be stored at 2–8℃ for 7–10 days.

IV. **Preparation of standard working solution:**

Before use, dilute the standard stock solution with double-distilled water at a ratio of 1:799 to prepare 25μg/mL (i.e., 200 U/mL) standard working solution, Prepare only the amount needed. It can be stored at 2–8℃ for 7–10 days.

Appendix I: Self-Contrast LZM Assay

Note: This method is method is suitable for samples of small number or turbid/coloured samples (such as blood red)

1. Operation procedure:

1. **Pre warm prepared bacteria working solution, standard working solution sample** 37℃ water bath for at least 5 minutes, make sure that these 3 liquids temperature reach to 37℃.
2. **Take test tubes (consider your sample number as n, you need to prepare standard tube and blank tube, so you need to take n+1+1 test tubes), place them** 37℃ water bath. Add 2mL bacteria working solution in each tube, place in water bath for longer time.
3. **Add distilled water in at least 2 cuvette, use them to adjust 100% at 530 nm on visible range spectrophotometer, remove distilled water.**
4. **Add 0.2mL standard working solution add 0.2 mL sample bottom of cleansed cuvette separately, then pour 2mL prewarmed bacteria working solution from test tubes in** 37℃ water bath to cuvette which contains 0.2 mL standard working solution or 0.2mL sample (you can use 5mL large transferpettor to transfer liquid). Use seconds-counter to count time when reaction starts, record transmissivity at 5 seconds as T_0 do not take cuvettes out of spectrophotometer, record transmissivity at 2 minutes 5 seconds as T_2.

   NOTE: After use a cuvette to assay 1 sample, please cleanse it by distilled water before add another sample or LZM standard solution.

5. Calculation Formula

$$\text{Lysozyme content}(\mu g/mL) = (UT_2 - UT_0 \div ST_2 - ST_0) \times C_S \times N$$

UT_0 is sample tubes transmissivity at 5 seconds after adding bacteria working solution.
UT_2 is sample tubes transmissivity at 2 minutes 5 seconds after adding bacteria working solution.
ST_0 is standard tubes transmissivity at 5 seconds after adding bacteria working solution.
ST_2 is standard tubes transmissivity at 2 minutes 5 seconds after adding bacteria working solution.
C_S is standard concentration (2.5μg/mL or express as 200U/mL), 2.5μg/mL= 2000U/mL, 1μg= 80U N is sample dilution times before assay

Appendix II: Blank-Contrast LZM Assay

Note: This method is suitable for limpid samples of large number.

1. Operation table

(It is suggested to operate in icewater bath)

	Blank tube (mL)	Standard tube (mL)	Test tube (mL)
Double-distilled water	0.2		
2.5 μg/mL standard working solution		0.2	
Sample (such as blood serum, urine, diluted saliva, etc.)			0.2
Bacteria working solution	2.0	2.0	2.0
Mix sufficiently, place in 37°C water bath for 15 minutes and place in an ice-water bath (below 37°C) for 3 minutes,transfer in cuvettes of 1cm light path tube, measure T_{15} of all tubes by at 530 nm (T_{15} is transmissivity at 15 minutes, adjust 100% by distilled water).			

Notes:

1. If there is water on cuvette surface, then please cleanse and dry it before measurement
2. In order to eliminate interference between sample tubes, then please rinse cuvette by distilled water before assay next tube.
3. OT_{15} is blank tubes transmissivity at 15 minutes, UT_{15} is sample tubes transmissivity at 15 minutes, ST15 is standard tubes transmissivity at 15 minutes.

2. Calculation Formula

$$\text{Lysozyme content}(\mu g/mL) = (UT_{15} - OT_{15} \div ST_{15} - OT_{15}) \times C_S \times N$$

Appendix III: Standard curve LZM Assay

1) Standard curve preparation

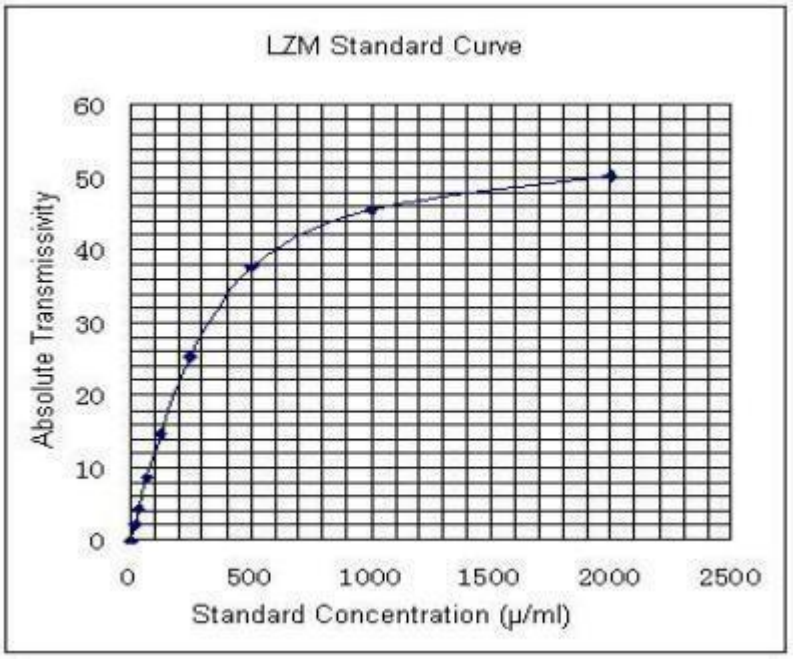
Preparation of standard solution:

1. Take 2 mg standard, add 1 mL of double-distilled water, mix sufficiently to prepare 160000U/mL standard stock solution. Dilute standard stock solution with distilled water to prepare standard solutions of different concentrations (such as 2000U/mL, 1000U/mL, 500U/mL, 250U/mL, 125U/mL, 62.5 U/mL, 31.25 U/mL, and 15.625 U/mL).
2. Precool bacteria working solution and LZM standard solution.

3. Operation table (it is suggested to use do this operation in icewater bath).

	Blank tube (mL)	Standard tube(mL)
Double-distilled water	0.2	
Standard solutions of different concentrations		0.2
Bacteria working solution	2.0	2.0
Mix sufficiently, place in 37℃ water bath for 15 minutes and place in an ice-water bath (below 37℃) for 3 minutes, transfer in cuvettes of 1cm light path tube, measure T_{15} of all tubes by at 530 nm (T_{15} is transmissivity at 15 minutes, adjust 100% by distilled water).		

4. **Draw standard curve:** Use standard concentration as x- axis, use transmissivity differences as y-axis, draw graph



2) Measure transmissivity as blank- contrast method, then you can get LZM content by checking graph (not as convenient as using formula)

Note : This kit includes standard, so it only needs to prepare 1-2 standard tubes per batch. You need not to draw standard curve by yourself in general.

3) Announcements

1. Grind the bacterial powder as finely and evenly as possible, and prevent splashing of the bacterial suspension during grinding.
2. The bacterial suspension, standard, and samples must be pre-cooled in an ice-water bath at 0℃ for more than 5 minutes. **Operations before the 37℃ water bath are preferably carried out in an ice-water bath.**
3. To avoid detection errors, use a separate cuvette for the blank tube. Before each sample addition or colorimetric measurement, rinse the cuvette with tap water first, then rinse with double-distilled water 2–3 times.
4. During colorimetric measurement, take each tube out of the ice-water bath one by one. An overly cold cuvette may form water mist on the wall, so wipe it dry before measurement.
5. Test tubes should be from the same batch.
6. To reduce errors, 1 cm path-length cuvettes should also be calibrated.