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 (5 mg/vial), pour in homogenate tube, add 1 mL bacteria powder diluent, grind by rotating slowly and softly for 3 minutes (do not splash out), bacteria stock solution is prepared. Prepared bacteria stock solution can be stored 2–8℃ hermatically for 1 week.

II. Bacteria working solution preparation:

Dilute bacteria stock solution with bacteria diluent at ratio 1:19. Residuary bacteria working solution can be stored at 2–8℃ for one week. Prepared working solution should be used soon after preparation, mixing sufficiently before use.

III. Preparation of standards:

- **Preparation of standard stock solution:**

Add 1.0 mL of distilled water to each vial of standard to prepare a 2 mg/mL standard stock solution, prepared stock solution can be stored at 2–8℃ for 7–10 days.

- **Preparation of standard working solution:**

When use, dilute standard stock solution with distilled water at a ratio of 1:799 to prepare 25 μg/mL (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 2 mL 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 0.2 mL standard working solution add 0.2 mL sample bottom of cleansed cuvette separately, then pour 2 mL prewarmed bacteria working solution from test tubes in** 37℃ water bath to cuvette which contains 0.2 mL standard working solution or 0.2 mL 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: sample tubes transmissivity at 5 seconds after adding bacteria working solution.

UT_2: sample tubes transmissivity at 2 minutes 5 seconds after adding bacteria working solution.

ST_0: standard tubes transmissivity at 5 seconds after adding bacteria working solution.

ST_2: standard tubes transmissivity at 2 minutes 5 seconds after adding bacteria working solution.

C_S: standard concentration (2.5 μg/mL or express as 200U/mL), 2.5 μg/mL= 2000U/mL, 1 μg= 80U

N: 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℃ 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).			

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. OT15 is blank tubes transmissivity at 15 minutes, UT15 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

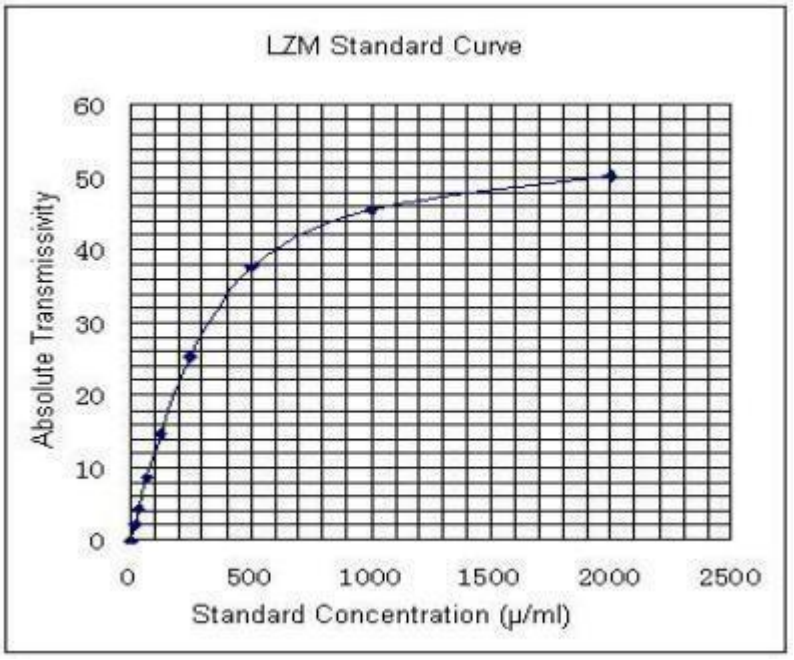
Preparation of standard solution:

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 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. 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. Bacteria powder should be grinded as fine as possible, avoid bacteria solution splashing out during grinding.

2. Bacteria solution, standard solution and sample must be precooled in 0℃ icewater bath for at least 5 minutes. It is suggested to operate in icewater bath before 37℃ water bath.

3. In order to avoid measuring error, please use 1 cuvette for blank tube separately. Before each adding and measuring, please cleanse cuvettes by tap water at first, then rinse them distilled water for 2-3 times.

4. Please dry cuvettes surface before measurement.

5. Please use test tubes in batch.

6. Adjust 1 cm light path cuvette to decrease error.

7. Time counting and transmissivity measuring should be at same time, control time accurately. It is suggest to use two operators or use automatic biochemical analyser.