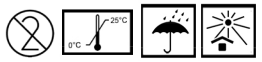
Endotoxin Assay Kit
Kinetic Turbidimetric Method
Instruction for use



REF: THS44-030
Product Name: Endotoxin Assay Kit, kinetic turbidimetric method
Specifications: 44tests/box
Intended use:
a) For quantitative detection of endotoxin on pharmaceuticals, biological products, radioactive medicine, medical device, hemodialysis solution, dialysis water, and ACD solution.
b) Other situation needs the quantitative detection of bacterial endotoxin.
Principle:
The clotting protein and clotting enzyme in the TAL(Tachypleus Amebocyte Lysate) could be activated by very small dose of endotoxin. Then TAL and bacterial endotoxin could agglutinate and come into gel. So it is through the Kinetic-Turbidimetric action to measure the Endotoxin.
Materials supplied with the Endotoxin Assay kit:

Item No.	Product Name	Description
THS44-030	Endotoxin Assay Kit (Kinetic-Turbidimetric method)	TAL(Kinetic-Turbidimetric) 0.3mL×44pcs; BET water 2mL×5pcs; Control Standard Endotoxin ×1pcs;

Materials required but not supplied:
Test tube rack, Vortex mixer, Laboratory Water Bath or dry bath, Endotoxin-free pipette tips, Adjustable Pipette, Laboratory film(Para film), Endotoxin-free tubes, 75% alcohol swab, etc.
All materials used for specimen collection and test reagent preparation must be free of interfering Endotoxin.
Instrument required:
BET-24A-48 tube reader with incubator, server
Reagent Storage:
Store all reagents, as supplied, at 0-25℃ in cool condition without corrosive gas and away from light, the related humidity should be less than 80%. The expiry date is 24 months. Use immediately after opening.
Procedures:
1. Generate standard curve:
Before testing, we must generate the standard curve or get Standard Curve Card from the supplier(with the card, do not need to generate standard curve, just used it as standard curve directly), for each batch of TAL, one standard curve needed.

1.1 Power the Laboratory dry or wet bath and BET-24A-48 tube reader on, thereafter, input the related information according to the user manual. Set up the temperature to 37℃, wavelength to 660nm. If have the standard curve ready, then for the same batch of TAL, just select the ready curve accordingly and go to step 2.
1.2 Preparation of the Control standard endotoxin solution
Reconstitute the Control Standard Endotoxin with BET water, the volume please refer to the CoA of CSE, and mix with Vortex mixer for 15min, then dilute it to 5.0EU/mL, 1.0EU/mL, 0.2EU/mL and 0.04EU/mL.
After reconstitution before any dilution, the CSE can be sealed and stored in refrigerator at -10C for 15days. When you need to use it, take it out and unfreeze it at room temperature and then Vortex for 15min.
For example:
If the CSE is 80EU/mL then dilute the CSE solution followed by below steps:
① Take out 0.2mL CSE solution of 80EU/mL + 1.4mL BET water, get 10EU/mL.
② Take out 1mL CSE solution from step ① + 1mL BET water, get 5EU/mL.
③ Take out 0.4mL CSE solution from step ② +1.6mL BET water, get 1.0EU/mL.
④ Take out 0.4mL CSE solution from step ③ + 1.6mL BET water, get 0.2EU/mL.
⑤ Take out 0.4mL CSE solution from step ④ + 1.6mL BET water, get 0.04EU/mL.
1.3 Preparation of the reagent:
Take out the 14pcs TAL, clean the bottle with 75% alcohol and open it by breaking apart it from the bottle neck.
1.4 Application of the sample:
Use BET water as negative control, it could be purchased separately.
Application of the samples to the TAL ampoules according to below table, then cover with Parafilm, and mix even on Vortex mixer, no bubble allowed.

Sensitivity of the Control Standard Endotoxin (EU/mL)	5.0			1.0			0.2			0.04			Negative	
	①	②	③	①	②	③	③	②	③	①	②	③	②	②
TAL(pcs)	1pcs	1pcs	1pcs	1pcs	1pcs	1pcs	1pcs	1pcs	1pcs	1pcs	1pcs	1pcs	1pcs	1pcs
BET water(mL)													0.3	0.3
CSE Solution (mL)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3		

Note: After Vortex, put the ampoule in the BET-24A-48 tube reader immediately, do not need to wait all sample application completed, just put into the tube reader right after the Vortex.

1.5 Test on the BET-24A-48 tube reader:

The tube reader will test and calculate automatically.

Applied Regression Analysis on the Log value of the reaction concentration (x) and the Log value of the reaction time (y), the standard curve is valid when meet the below requirements:

a) The testing time of the negative control should be more than the lowest value of the standard curve.

b) The absolute value of the correlation coefficient of the standard curve (r) should be bigger than or equal to 0.98.

$$r=\frac{\Sigma(X-\overline{X})(Y-\overline{Y})}{\sqrt{\Sigma(X-\overline{X})^2\cdot\Sigma(Y-\overline{Y})^2}}$$

According to LgT=a+bLgC, we get the standard curve.

1.6 Using the standard curve as the calibrator.

2 Quantitative testing for the samples:

2.1 The preparation of the sample:

Calculate Maximum valid dilution(MVD)= cL/λ

c: If the unit of the endotoxin limit is EU/mL, then c=1

L: Endotoxin limit

λ: Lowest point of the standard curve (EU/mL)

Example: If the sample is 10% glucose, the endotoxin limit is 0.5EU/mL, and the lowest point of the standard curve is 0.03EU/mL:

MVD=cL/λ=1×0.50/0.03=16

Then the Maximum valid dilution is 16.

For example:

1) take out 0.2mL 10% glucose, add 1.4mL BET water, we get the dilution rate of 8
2) take out 1mL from above solution and add 1mL BET water to it, we get the dilution rate of 16.

2.2 The preparation of the positive product control solution:

Put 0.5mL Control Standard Endotoxin (1.0EU/mL) and 0.5mL sample solution with the dilution rate of 8 together in an endotoxin-free tube and mix with Vortex.

2.3 Take out TAL, clean the bottle with 75% alcohol and open it by breaking apart it from the bottle neck.

2.4 Application the samples to TAL ampoules according to below table, then cover with Parafilm and mix for 3s on Vortex mixer, no bubble allowed:

Reagent \ tubes	Sample tube		Negative control		positive product control	
	1	2	1	2	1	2
TAL(pcs)	1pcs	1pcs	1pcs	1pcs	1pcs	1pcs
BET water(mL)			0.3	0.3		
Sample(mL)	0.3	0.3				
PPC solution(mL)					0.3	0.3

Notes:

①Since the standard curve generated by the same lot of the TAL, so there is no need to do the positive control.

② After Vortex, put the ampoule in the BET-24A-48 tube reader immediately, do not need to wait all sample application completed, just put into the tube reader right after the Vortex.

2.4 BET tube reader will test and calculate automatically.

If the result of Sample tube times the dilution ratio less than the requirement, then the sample is qualified.

If the result of Sample tube times the dilution ratio equal with or more than the requirement, then the sample is failed.

Precautions:

1. All materials used for specimen collection and test reagent preparation should be endotoxin-free.
2. The pH value of the specimen should be within 6-8, if not, the pH buffer should be used. Always measure the pH of the bulk sample to avoid contamination by the pH electrode. pH buffer could be purchased separately.
3. If there is no result from the test, then please calculate using zero.
4. If interference test is necessary, please refer to National Pharmacopeia.
5. Use TAL with same lot number for one test.
6. For the Control Standard Endotoxin solution, after reconstitution before any dilution, the CSE can be sealed and stored in refrigerator at -10C for 15days. When you need to use it, take it out and unfreeze it at room temperature and then Vortex for 15min.
7. Do not need the positive control when standard curve generated before testing. Otherwise, the positive control is a must.
8. Only one standard curve needed for the same lot.
9. Specimens should be clearly labeled during the test.
10. The TAL(Tachypleus Amebocyte Lysate), which occasionally causes some people sneezing and/or watery eyes symptoms of allergy after contact. Please stop the operation and move to ventilation and open place if such uncomfortable symptoms occur.

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