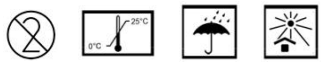
Endotoxin Assay Kit

Instruction for use



REF: TVG96-0250, TVG96-0125, TVG96-0060, TVG96-0030

Product Name: Endotoxin Assay Kit, Gel-Clot

Specifications: 96 tests/box

Intended use:

- For the qualitative detection of endotoxin with gel-clot method on pharmaceuticals, biological products, radioactive medicine, and medical device.
- For the pyrogen control or endotoxin detection during the production.
- Other situation needs detection of endotoxin with gel-clot method.

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 an agglutination to detect the endotoxin by qualitation.

Materials supplied with the Endotoxin Assay kit:

Item No.	Product Name	Description
TVG96-0030	Endotoxin Assay Kit (Gel-Clot)	TAL(0.03EU/mL) 2.4mL×4pcs; BET water 8mL×4pcs Control Standard Endotoxin ×1pcs;
TVG96-0060	Endotoxin Assay Kit (Gel-Clot)	TAL(0.06EU/mL) 2.4mL×4pcs; BET water 8mL×4pcs Control Standard Endotoxin ×1pcs;
TVG96-0125	Endotoxin Assay Kit (Gel-Clot)	TAL(0.125EU/mL) 2.4mL×4pcs; BET water 8mL×4pcs Control Standard Endotoxin ×1pcs;
TVG96-0250	Endotoxin Assay Kit (Gel-Clot)	TAL(0.25EU/mL) 2.4mL×4pcs; BET water 8mL×4pcs Control Standard Endotoxin ×1pcs;

Materials required but not supplied

Vortex mixer; Laboratory Water Bath (incubator, the temperature could be set to 37±1℃); Test tube rack, Adjustable Pipette, Laboratory Film (Parafilm), 75% alcohol swab, endotoxin-free tube and endotoxin-free pipette tips.

Note: endotoxin-free tube and endotoxin-free pipette tips could be purchased separately.

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. Check the sensitivity of TAL if necessary

1.1 Preheat the incubator to 37±1℃

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, mix for 15min with Vortex mixer, as per the sensitivity(λ) of the TAL dilute it to 2λ, λ, 0.5λ and 0.25λ, mix with Vortex mixer for 30s for each step. I.e. λ=0.125EU/mL, then dilute the control standard endotoxin to 0.25EU/mL, 0.125EU/mL, 0.0625EU/mL and 0.0312EU/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.

1.3 Preparation of the TAL solution and application of the samples.

Take out TAL, clean the bottle neck with 75% alcohol and open it, add 2.4mL BET water, mix with Vortex mixer for 2-3s. put 18pcs endotoxin-free testing tubes on the test tube rack, and application samples according to below table:

Sensitivity of the endotoxin	0.25EU/ml				0.125EU/ml				0.0625EU/ml				0.0313EU/ml				Negative Control	
TAL(mL)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BET water (mL)																	0.1	0.1
CSE solution (mL)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

After application the samples, mix them even with Vortex mixer, cover the tube with laboratory film(Parafilm), and put them into incubator which setting the temperature at 37±1℃. Last for 60min±2mins. Then carefully remove the tubes one by one from the incubator. Gently invert the tubes to 180 degrees, if a solid clot has formed the result is positive (+), if no clot has formed, the result is negative (-). Do not shake the tubes when reading the test.

The test is valid only when:

Both negative tubes: no clot has formed (-)

0.25λ tubes: no clot has formed (-)

2λ tubes: Should clot (+)

Calculate the sensitivity of the TAL(λc) via below formula:

$\lambda c = lg^{-1}(\sum X/4)$

X =lg (highest sensitivity appeared to be positive (+))

Only when the value of λc is between 0.5λ to 2λ, the TAL could be used for testing, and please use the nominated sensitivity. I.e. according to the test result in below table,

	0.25(2λ)	0.125(λ)	0.0625(0.5λ)	0.0312(0.25λ)	Negative control	highest sensitivity appeared to be (+)
1	+	+	-	-	-	0.125
2	+	+	-	-	-	0.125
3	+	+	-	-		0.125
4	+	+	-	-		0.125

$\lambda c = lg^{-1}(\sum X/4)$

$= lg^{-1}((lg0.125+lg0.125+lg0.125+lg0.125)/4)$

=0.125EU/mL

2. Sample testing

2.1 Preheat the incubator to 37±1℃

2.2 Preparation of the positive control solution:

Reconstitute the Control Standard Endotoxin with BET water, the volume please refer to the CoA of CSE, mix for 15min with Vortex mixer and dilute it to 2λ and 4λ (λ is the sensitivity of the TAL).

Example: If the sensitivity of the Control standard endotoxin is 10EU/mL, and λ=0.125EU/mL then please follow the below steps:

$$10EU/ml \xrightarrow[1.8ml]{0.2ml} 1.0EU/ml \xrightarrow[1.0ml]{1.0ml} 0.50EU/ml \xrightarrow[0.5ml]{0.5ml} 0.25EU/ml$$

Above volume is the amount being taken out, while the below volume is the amount we add BET water.

2.3 Preparation of the sample:

Calculate Maximum valid dilution (MVD)= cL/λ

L: Endotoxin limit

c: Density of the sample, when the unit of L is EU/mL, then c=1.0mL/mL; when the unit of L is EU/mg or EU/U, then the unit of c should be mg/mL or U/mL;

λ: Sensitivity (EU/mL) of TAL

Example: If endotoxin limit is 0.5EU/mL, the sample is glucose injection, λ=0.125EU/mL then MVD=1.0*0.50/0.125=4

Then take out 0.50mL sample and put into 1.50mL BET water, mix even.

Note: 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

2.4 Preparation of the positive product control solution:

Mix the 0.5mL Control standard endotoxin solution (4λ) and 0.5mL 2 times diluted sample solution (if it is glucose injection, take out 0.25mL and add 0.25mL BET water, mix even) with Vortex mixer.

2.5 Preparation of the reagent:

Take out TAL, clean the bottle neck with 75% alcohol and open it, add 2.4mL BET water, mix with Vortex mixer for 2-3s.

2.6 Application of samples: Put 8pcs endotoxin-free tubes on the test tube rack. Refer to the table below for application of samples and mix even by Vortex.

	Sample tube		Negative control		Positive product control		Positive control	
	①	②	①	②	①	②	②	②
TAL(mL)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BET water			0.1	0.1				
Samples	0.1	0.1						
Control standard endotoxin solution(2λ)							0.1	0.1
Positive product control solution					0.1	0.1		

2.7 Cover the tubes with Laboratory Film, then put it into the 37℃±1 incubator for 60±2mins.

2.8 Then carefully remove the tubes one by one from the incubator. Gently invert the tubes to 180 degrees, if a solid clot has formed the result is positive (+), if no clot has formed, the result is negative (-). Do not shake the tubes when reading the test.

2.9 Interpretation of the result:

The test is valid only when:

Both negative tubes: no clot has formed (-)

Both Positive product control tubes: should clot (+)

Both positive control tubes: Should clot (+)

The sample tubes:

If both negative (-) means the sample contains an endotoxin concentration less than λ*MVD; If both positive (+), means the sample contains an endotoxin concentration of at least λ*MVD. If one is negative (-), and the other is positive (+), then retest the sample. And 4 sample tubes needed, all 4 are negative means qualified, else not qualified.

Precaution

1. The pH value of the sample solution 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.
2. All materials used for specimen collection and test reagent preparation should be endotoxin-free.
3. Mix always with the Vortex mixer.
4. Do not shake the tubes when reading the test.
5. If interference test is necessary, please refer to National Pharmacopeia.
6. Specimens should be clearly labeled during the test.
7. Main reagent is made from lyophilized 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.

Reference

1. Kun Wang, The application value of detect of bacterial endotoxin in patients with infectious disease in ICU ward. China Modern Medicine. 2014, 01(b)-0125-02.
2. Van Deventer SJ, Buller HR, ten Cate JW, et al. Endotoxaemia: an early predictor of septicaemia in febrile patients[J]. Lancet, 1988, 1(9596):605-609.
3. Ling Wang, Guiping Wang. Endotoxin detecting method and the application summary[J]. China Pharmacist, 2003, 6(5): 316-317.
4. Annex II E Bacterial Endotoxins Test (Kinetic-Turbidimetric method). 2010

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