Endotoxin Removal Report

Catalog Number: AAA18545

1. Preparation for Experiment Supplies

Endotoxin work product; LAL (Sensitivity 0.25EU / ml, purchased from the following TAL manufacturer); Bacterial endotoxin test water (BET water); Heat source removed centrifuge tube; Vortex shaker; 37℃ Incubator or thermostatic water bath; Timer; Imported sterilization pipette tip.

Heat source removed centrifuge tube (1.5ml): filled with 0.1M NaoH, soaked for more than 4 hours, the remaining liquid was drained to dry, washed with BET water for 4 times

Use imported sterilization pipette tip during all column and sample dilution process.

2. Removal of Protein Endotoxin

2.1. Install the column for endotoxin removal, and then install drainage tube, and wash off the alcohol in the column with water.
2.2. Add sodium deoxycholate and wash column for 3 times
2.3. Wash column for 3 times using bacterial endotoxin testing water, and pull out the drainage tube after finishing washing, and let the water endotoxin go.
2.4. Add the protein containing endotoxin, under the drainage tube, use the heat source removed centrifuge tube to drainage the protein left in the tube. After most protein in the column were left, add 100ul of endotoxin testing water, and keep catching the remaining protein, and repeat for 5 times.
2.5. Wash the column with endotoxin water and then seal the column with 20% ethanol, standby.

3. Preparation for LAL

Open the LAL bottles, and add 0.1ml BET water into each bottle, and gently shake to make LAL completely dissolved. Be careful not to cause air bubbles.

4. Preparation for Protein Dilution

Put the endotoxin removed protein in sterile operation platform, in heat source removed centrifuge tube, dilute the protein to be measured with 400, 800, 1200, 2000, 4000 dilution ratios using BET water.

5. Preparation for Gel Reaction Tube

Negative control: 0.1ml LAL+0.1ml Endotoxin testing water

Protein reaction tube: 0.1ml LAL+0.1ml 400x Diluted protein
0.1ml LAL+0.1ml 800x Diluted protein
0.1ml LAL+0.1ml 1200x Diluted protein
0.1ml LAL+0.1ml 2000x Diluted protein
0.1ml LAL+0.1ml 4000x Diluted protein

6. Incubation of Reaction Tube

Fix the reaction tube vertically and incubate for 60min (+/-2min) at 37℃. Avoid shaking during take up, put down and incubation process. Otherwise, it will seriously affect the gel formation result.

7. Result Interpretation and Calculation

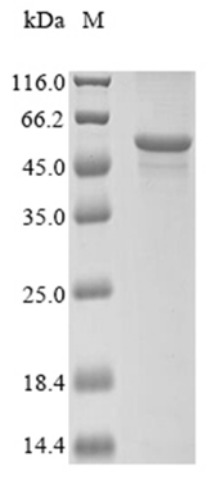
7.1. Gently take out the reaction tube from the thermostat, and reverse 180 degrees slowly. If the content in the tube shows solid gel, no deformation, does not slip off the wall, then it is positive and record as (+). If the content in the tube is not a gel or can't remain intact after form a gel, or slip off the wall, then it is negative and record as (-).

7.2. The Experiment Result for this Time:

	Protein Dilution Ratio				
Negative	**400**	**800**	**1200**	**2000**	**4000**
(-)	(+)	(+)	(+)	(-)	(-)

7.3. Protein Concentration Result after Removing Endotoxin

MBS1004050: 0.5mg/ml (by the Bradford Method), 500ug/ml



7.4. Calculation Result for Endotoxin Content

Endotoxin Content = (0.25 EU /ml *1200) /(500ug/ml) =0.6EU/ug

(The general endotoxin requirement for recombinant protein commodity ≤1EU/ug)

Meets the requirement.