

# MEBEP TECH(HK) Co., Limited

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# **Endotoxin Removal Beads**

**Product Number: ERB01** 

## **Shipping and Storage**

Store at 2-8°C.

### **Description**

Endoxin RemovalLeads is a product used to remove endotoxins from biologically derived protein products, including peptides, antibodies, polysaccharides, etc. It involves attaching modified polymyxin B onto 4% agarose microspheres for specific removal of endotoxins, which can reduce the endotoxin content in the sample to 0.1 EUm and achieve high sample recovery rate.

#### **Specification**

1. Matrix: 4% agarose microspheres

2. Ligand: Modified polymyxin B

3. Load capacity: >2.000000EU/mL medium

4. Particle size: 45-165μm

5. Maximum flow velocity: 0.1MPa, 1 bar

6. PH stability range: 5-10

7. Tolerant reagents: 20% DMSO, 20% Z-alcohol, 20% glycerol; 1M urea, 300mM urea; 0.05% Twean20, 10mMDTT, etc.

8. Storage buffer solution: 1XPBS containing 20% ethanol

#### **Protocol**

#### 1. Preparation of buffer solution

All water and consumables must be heat free products to prevent the introduction of endotoxins during use.

Equilibrium solution: 20mM phosphate, 0.15M NaCl, pH 7.4.

Regeneration solution: 1% Tritonx-114 equilibrium solution.

Note: The equilibrium solution and eluent can be adjusted according to the properties of the sample. It is recommended to have a pH of 7-8 and a NaCl concentration of approximately 0.15M-0.5M.

## 2. Sample preparation

It is recommended to centrifuge or filter the sample with a  $0.22\mu m$  or  $0.45\mu m$  filter membrane before loading to reduce impurities, improve protein purification efficiency, and prevent column blockage.

It is best to control the pH of the sample at pH 7-8, as the optimal pH for endotoxin binding to the column is 6-9.

It is best to control the appropriate ion strength of the sample to reduce non-specific adsorption, such as 0.15-0.5M NaCl.

#### 3. Endotoxin removal

- 3.1. Mix Endoxin Removal Leads thoroughly, use a non heat source gun to extract an appropriate amount of slurry (taking 1mL as an example) and add it to the chromatography column. Open the lower outlet to remove the protective solution. Clean with 3m regeneration solution, control the flow rate at 0.25mL/min or less than 10 drops per minute, and maintain the temperature between 2-8°C. Repeat at least twice to ensure that there are no endotoxins in the column.
- 3.2. Use 3mL of equilibrium solution to balance the inner wall and packing of the column tube, let it dry, flow at a rate of about 0.5m1min, control the temperature at 2-8°C, and repeat at least twice.
- 3.3. Add the sample to the balanced Endotoxin RemovalBeads, and adjust the flow rate to 0.25mL/min, or less than 10 drops per minute. When the outflow flows out about 1mL, start to collect the outflow, and add 1mL of equilibrium solution to continue to collect after the outflow dries up. Detect the endotoxin content and sample recovery rate in the sample.
- 3.4. If the endotoxin content in the sample is still higher than the target value, continue to repeat steps 3.1-3.3.



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## **Question and Solution**

Question	Analysis of causes	Solution
Low efficiency of endotoxin removal	The pH value of the sample is not	Adjust pH 7-8 with 0.1 M NaOH or
	within the range of endotoxin binding	0.1M HCl.
	Short contact time between sample and	Reduce flow rate and increase sample
	filler	contact time.
	Remove or detect system	All experimental supplies must be heat
	contamination by endotoxins	free products.
Low efficiency of endotoxin removal	Strong binding between endotoxin and	Optimize the pH of the sample to
	target protein	separate it from endotoxins.
		Ask when increasing contact
The sample is contaminated	Purified fillers for other samples	Do not use used fillers to remove
		endotoxins from different samples.
Low sample recovery rate	Non specific adsorption of samples on	Increase the concentration of NaCl in the
	fillers	sample and equilibrium solution.
	The target protein is removed together	Optimize the pH of the sample to
	with endotoxin binding	separate it from endotoxins.