Endotoxin Removal Kit

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1. Product Description

Endotoxin Removal Beads are used to remove endotoxin in biological protein products (including polypeptides, antibodies and polysaccharides). The modified polymyxin B are attached to 4% agarose for specific removal of endotoxin. The endotoxin in the sample is reduced to 0.1EU/ml, with a high protein recovery rate.

Table 1. Characteristics of **Endotoxin Removal Beads**

Item	Description
Matrix	4% agarose
ligand	Modified polymyxin B
Binding capacity	>2,000,000EU/ml medium
Particle size（μm）	45-165
Maximum pressure	0.1 MPa, 1 bar
pH stability	5-10
Chemical compatibilities	20% DMSO,20% ethanol,20% glycerinum,1M urea,300mM imidazole；0.05% Tween 20,10mM DTT
Storage solution	20% ethanol
Storage	2°C - 8°C

Table 2. The component of **Endotoxin Removal Kit**

	Size	Amount
Endotoxin Removal Column	1.5ml	1
Regeneration Buffer	125ml	1
Binding Buffer	125ml	1

2. Endotoxin Removal Procedure

2.1 Buffer Preparation

Please use endotoxin-free water and consumables to prevent the introduction of endotoxin during sample purification.
Binding Buffer and Regeneration Buffer are provided.
The Regeneration Buffer is a slightly cloudy liquid at room temperature. If a solid substance is found, the Regeneration Buffer can be placed in a 2-8 ℃refrigerator or on ice and shaken intermittently until become a homogeneous and transparent solution.
Note: The Binding Buffer can be changed according to the properties of the samples. It is suggested that pH 7-8 and NaCl is about 0.15M-0.5M.

2.2 Sample Preparation

It is recommended to filter the sample solution by passing them through a 0.22μm or 0.45 μm filter before use to reduce impurities, improve protein purification efficiency and prevent clogging of the column.
The pH of the sample is best controlled between pH 7-8, because the optimum pH for endotoxin binding to the beads is 6-9.
It is better to control the appropriate ion strength of the sample to reduce non-specific adsorption, such as 0.15-0.5M NaCl.

2.3 Endotoxin removal

1) Fix Endotoxin Removal Column and remove the bottom plug and top plug. Allow storage buffer to drain from resin by gravity flow.
2) Regenerate column with 5ml of cold Regeneration Buffer with endotoxin-free tips. Control the flow rate at 0.25ml/min, or less than 10 drops per minute. Allow buffer to drain from the column. Repeat this step two times. It is recommended to control the temperature of 2-8℃ during regeneration.

3) Add 6ml Binding Buffer evenly along the inner wall to equilibrate column. Allow buffer to drain from the column. Adjust the flow rate to 0.5ml/min. Repeat this step two times. It is recommended to control the temperature at 2-8℃ during balance.
4) Apply the pre-treated sample to the column and collect the flow-through after initial 1.5ml. Control flow rate at 0.25ml/min, or less than 10 drops per minute. Add 1.5ml Binding Buffer onto the column after entire sample enter the resin.
5) Determin the contents of endotoxin and recovery of samples.

6) If endotoxin level is still higher than the target value, repeat the procedure.

3. Troubleshooting

Problem	Probable cause	Solution
Endotoxin removal is inefficient	The pH value of the sample is not within the endotoxin binding range	Adjust pH to 7-8 with 0.1MNaOH or 0.1M HCl.
	Short contact time between sample and resin	Reduce flow rate and increase sample contact time.
	Remove or detect system contaminated with endotoxin	All System should be endotoxin-free.
	Endotoxin strongly binds to target protein	The pH of the sample was optimized to separate the sample from endotoxin. Increase contact time.
Contaminated sample	Resin was used for other samples	Do not reuse resins to remove endotoxin for different samples.
Low sample recovery	The sample was nonspecific adsorbed on the resin	Increase the concentration of NaCl in the sample and equilibrium solution.
	The target protein is removed in combination with the endotoxin	The pH of the sample was optimized to separate the sample from endotoxin.

4. Related Products

Product	Cat. No.	Size
Endotoxin Removal Beads	SA031005	5 ml
	SA031025	25 ml
	SA031100	100 ml
	SA031500	500 ml
	SA03101L	1 L
Endotoxin Removal Kit	SA031K03	3 Assays