Protocol for PurKine™ Endotoxin Removal Products

Item NO.	Product Name
BMR21400	PurKine™ Endotoxin Removal Resin
BMC21400	PurKine™ Endotoxin Removal Packed Column
KTP21400	PurKine™ Endotoxin Removal Kit (polymyxin B)

ATTENTION

For laboratory research use only

Not for clinical or diagnostic use

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Reagent Preparation

It is recommended to use only new plasticware that is certified to be pyrogen-or endotoxin-free. Regeneration buffer is slightly turbid at room temperature. Please shake gently. If it exists solid substance, place regeneration buffer at 2-8°C or on the ice. Intermittent shaking, until it shows uniform, transparent.

Regeneration buffer：1% Triton X-114
Equilibration buffer：20mM NaH_2PO_4, 0.15M NaCl, pH7.4

Sample Preparation

The sample should be centrifuged and/or filtered through a 0.22μm or 0.45 μm filter before it is applied to the medium to prevent clogging the column. It is recommended the PH of sample is pH 7-8, because the best PH for endotoxin binding to the column is PH 6-9. Keep the sample in appropriate ionic concentration to reduce nonspecific adsorption, such as 0.15-0.5 M NaCl.

Protocol for Sample Purification

Note: Regenerate the resin before the first use and after each subsequent use. Equilibrate all solutions and the resin to room temperature before use.

1. Place the column upright in the stand. Remove the top cap first to prevent bubbles from being drawn into the gel. Allow storage solution drain completely from the column, but do not allow the column bed to dry.
2. Wash the column by adding 5 resin-bed volumes of cold regeneration buffer (Do not warm it up, otherwise it will become cloudy) and let the buffer drain completely. Set the flow rate at 0.25 ml/min or at most 10 drops per minute by adjusting the flow speed. Repeat the wash step two more times to make this system endotoxin-free. It is important to rinse the wall of the column from top to bottom using regeneration buffer.
3. Equilibrate the column by adding 5 resin-bed volumes of equilibration buffer and let the buffer drain completely at a speed of 0.5 ml/min. Also, the column wall should be rinsed completely during this process. Repeat the equilibration step two more times.
4. Close the flow-speed control after column equilibration. Add sample to the column. Set the flow rate at 0.25 ml/min or at most 10 drops per minute by adjusting the flow-speed. Start collecting the sample eluate with endotoxin-free tube until the volume of eluate is up to 1.5 ml. In order to reduce the loss of sample, it's recommended rinsing again with 2 resin-bed volumes of equilibration buffer after all the sample completely gets in the column. Repeat one more time. Pool the fractions containing protein sample and detect the endotoxin in it.
5. Reloading of the Sample. If the final endotoxin level is above the desired endotoxin level. Repeat the endotoxin removal procedure by reloading the sample to the regenerated column.
6. Storage of the Column. For storage of the column, wash the column with 5 resin-bed volumes of equilibration buffer and allow the column to drain completely. Add 1 resin-bed volume of regeneration

buffer (contain 20% ethanol). Store at 2°C to 8°C. Do not freeze.

Troubleshooting

Problem	Probable cause	Solution
Low endotoxin removal efficiency	Sample pH was not within endotoxin binding range	Adjust sample to pH 7-8
	Incubation time was not sufficient	Reduce flow speed
	The removal or detection system was contaminated by extrinsic LPS	Use endotoxin-free labware and buffers
	Endotoxin was bound to the target protein	Recycle the sample through the column several times
Sample contamination	Different samples were purified by the same resin	Avoid purifying different samples using same resin
Low protein/sample recovery	Target protein aggregated with endotoxin and was removed	Increase NaCl concentration in the sample and equilibration buffer to 0.5M
	Nonspecific binding of sample to the resin	