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PurKine™ Endotoxin Removal Kit (Polymyxin B)

Cat #: KTP2140 Size: 1 mL/1 mL×5

	Endotoxin Removal Kit (Polymyxin B), crosslinked 4% agarose		
REF	**Cat #:** KTP2140	LOT	**Lot #:** Refer to product label
	Capacity: 2,000,000 EU/mL		**Bead size:** 45-165 μm
	Tolerance: 0.1 MPa, 1 bar		**Buffer:** Pyrogen/Endotoxin-free
	Storage: Stable for 12 months at 4℃		**Note:** Use endotoxin-free plasticware and water

Assay Principle

Endotoxin is the lipopolysaccharide (LPS) complex located in the outermembrane of Gram-negative bacteria. A single E.*coli* contains 2 million molecules LPS (2-20 fg/cell). During experimental procedures, large amount of endotoxins can easily contaminate consumables, buffers, samples and other downstream products. In vitro, endotoxin causes a variety of problems in cell-culture and cell-based research. In vivo, endotoxin may cause various side effects, including inflammatory response, organ failure or septic shock in host organisms. PurKine™ Endotoxin Removal Kit can quickly and effectively eliminate endotoxins to <0.1 EU/mL in solutions containing proteins or pharmacologically important components via the immobilized Polymyxin B, which is known for capturing endotoxin and preventing toxic effects.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	1 mL	1 mL×5	
PurKine™ Endotoxin Removal Packed Column	1 mL	1 mL×5	4℃
Regeneration Buffer (10×)	15 mL	75 mL	4℃
Equilibration Buffer (10×)	30 mL	100 mL+50 mL	4℃

Materials Required but Not Supplied

- 0.22 μm or 0.45 μm filter
- Precision pipettes, disposable pipette tips
- Pyrogen or endotoxin-free water
- Various glassware for preparing reagents and buffer solutions

Sample Preparation

The sample should be centrifuged and/or filtered through a 0.22 μm 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.

Reagent Preparation

Water, buffer and consumables are to be pyrogen-free.

Equilibration buffer: Equilibration Buffer (10×) was diluted with deionized water to Equilibration Buffer before use, store at 4℃.

Regeneration buffer: Regeneration Buffer (10×) was diluted with deionized water to Regeneration Buffer before use, store at 4℃.

Note: Equilibration buffer may be changed depending on sample properties and NaCl, pH 7-8 around 150 mM -500 mM is recommended.

Procedure 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 min 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 min 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.

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. Store at 2℃ to 8℃. Do not freeze.

Trouble Shooting

Problem	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 pyrogen/endotoxin-free ware and water
	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 to 500 mM
	Nonspecific binding of sample to the resin	

Recommended Products

Catalog No.	Product Name
BMR2140	PurKine™ Endotoxin Removal Resin
KTP2001	PurKine™ His-Tag Protein Purification Kit (Ni-NTA)
KTP2010	PurKine™ GST-Tag Protein Purification Kit (Glutathione)
KTP2020	PurKine™ MBP-Tag Protein Purification Kit (Dextrin)
KTP2030	PurKine™ Biotin-Tag Protein Purification Kit (Streptavidin)
KTP2070	PurKine™ Antibody Purification Kit (Protein A/G)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.