EndoTrap® 5/1

Endotoxin removal system

Introduction

EndoTrap is a brand new affinity matrix for the efficient removal of bacterial endotoxins from solutions. EndoTrap can be employed both in batch or chromatography mode. EndoTrap has been developed for the removal of endotoxins from aqueous solutions containing low or high molecular weight substances. Frequently, endotoxin removal from protein solutions is insufficient with standard methods including ultrafiltration, ion exchange chromatography, or two phase extraction.
The high affinity of EndoTrap ligand to endotoxin enables the efficient capturing of endotoxins even at very low endotoxin concentrations. The EndoTrap ligand is immobilized covalently on beaded agarose to ensure negligible leakage of EndoTrap ligand. The endotoxin binding capacity of EndoTrap in aqueous buffers is about 2 x 10^6 EU/ml matrix. Non specific binding of proteins to EndoTrap is extremely low, delivering a mass yield which typically exceeds 95 %. The EndoTrap system can be reused at least 3 times without loss of endotoxin removal efficiency!

Kit Components

Prepacked EndoTrap columns	5 pcs.
Equilibration buffer (EB) [1]	2 x 125 ml (**recipe see footnote**)
Regeneration buffer (RB)	125 ml

Storage

EndoTrap is supplied as prepacked columns (EndoTrap 5/1, Cat.Nr.: 311063, 1 ml column material in 20% ethanol) or as a 50% slurry in regeneration buffer (EndoTrap 10, Cat.Nr.: 311064 or EndoTrap 100, Cat.Nr.: 311065). EndoTrap is stable for at least 4 weeks between 4°C and 25°C. Regenerated EndoTrap matrix should be stored at 4°C in regeneration buffer (RB) supplemented with 0.02% sodium azide or in 20 % ethanol. Do not freeze.

Material Not Provided

Storage buffer	Regeneration buffer with 0.02 % sodium azide or supplement 20% ethanol

Precautions

- ! **All used materials like containers or pipette-tips and buffers must be endotoxin free.** Glass ware is preferred, as endotoxins can be removed by heat treatment (200°C, 4h; 250°C, 1h).
- ! **Buffers used for endotoxin removal with EndoTrap have to contain at least 50 µM Ca^{2+}.**
- ! Buffers should be prepared from endotoxin free materials and endotoxin free water.
- ! When using EndoTrap columns, all buffers including equilibration buffer EB and regeneration buffer RB should be degassed prior to use. When using EndoTrap gel slurry, degas slurry prior to use – see FAQ for details.
- ! Avoid proteases and organic solvents.

[1] **Equilibration buffer (EB):** 20 mM Hepes, 150 mM NaCl, 0.1 mM $CaCl_2$, pH 7.5

Specifications

Binding capacity	up to 2.000.000 EU / ml matrix
Ligand density	8 mg ligand / ml matrix
pH stability	pH 3-9
Support matrix	spherical, cross-linked agarose
Void volume	0,3 to 0,5 ml
Mean particle size	90 µm
Storage	at 4°C in regeneration buffer (RB) supplemented with 0.02% sodium azide or in 20% ethanol

Protocols

EndoTrap can be used either in batch or column mode. In general, endotoxin removal of high endotoxin levels is more practical in the column mode while low endotoxin levels are more efficiently removed in batch processing. However, parameters such as pH, ionic strength, temperature, contact time etc. might have to be optimized for each application to obtain maximum endotoxin removal with minimum loss of product.

Column Mode

Preparation

1. To use a prepacked column, remove the top cap first. This prevents bubbles from being drawn into the gel. Next, remove end cap and place the column in a suitable holder. Allow storage solution to drain completely from column.
2. If you use EndoTrap gel slurry, fill the slurry in an appropriately sized column and allow gel to settle for 30 minutes.

Activation and Endotoxin Removal

1. Wash column with 6 column volumes of regeneration buffer (RB).
2. Equilibrate the column with 6 column volumes of equilibration buffer (EB).
3. Apply sample onto the column, start collecting immediately. Let sample drain completely from column.
4. Add 6 volumes of equilibration buffer (EB). Collect all. A fraction size of 1 ml is recommended.

Regeneration and Storage

1. If you want to store the column, allow the equilibration buffer (EB) to drain completely from column. Apply 1 ml of regeneration buffer (RB) supplemented with 0.02% sodium azide and store at 4°C. Instead of regeneration buffer (RB) and sodium azide you can use 20% ethanol.
2. Prior to the next run make sure you start with step 1 of Activation and Endotoxin removal.

Batch Mode

All centrifugation steps should be carried out at 1.200 x g for 2 min at room temperature!

Preparation

1. Remove storage buffer from gel slurry by centrifugation and aspirate the supernatant.

Activation and Endotoxin removal

1. Add 2 gel volumes of regeneration buffer (RB), mix by gently shaking the tube for 5 sec.; centrifuge, and aspirate the supernatant. Repeat this step 2 times.
2. Add 2 gel volumes of equilibration buffer (EB), mix by gently shaking the tube for 5 sec.; centrifuge and aspirate the supernatant. Repeat this step 2 times.
3. Add the sample (1 ml to 10 ml of your protein sample) and incubate for at least 30 minutes at RT. Gently rock or rotate the tube while incubating.
4. Centrifuge at 1.200 x g for 5 minutes and transfer the supernatant to an endotoxin free tube.

Regeneration and Storage

1. If you want to store the EndoTrap Gel, resuspend the EndoTrap Gel pellet in 1 volume of regeneration buffer RB supplemented with 0.02% sodium azide and store at 4°C. Instead of regeneration buffer RB and sodium azide you can use 20% ethanol.
2. Prior to the next run make sure you start with step 1 of “Activation and Endotoxin removal”.

Our Related Products

ORDER INFORMATION		
Product	**Contents**	**Cat. No.**
EndoTrap 5/1	5 x 1 ml columns, ready to use, equilibration buffer, regeneration buffer	311063
EndoTrap 10	10 ml resin (50% slurry), equilibration buffer, regeneration buffer	311064
EndoTrap 100	100 ml resin (50% slurry), equilibration buffer, regeneration buffer	311065
EndoTrap C	Column resin, bulk size available	311066
Regeneration buffer	125 ml	311067
Equilibration buffer	125 ml	311108
Empty columns	0,2 ml to 10 ml columns	311068

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