Miltenyi Biotec

Endotoxin Removal Beads

3 mL	130-093-657
5×3 mL	130-093-659

Contents

1. Description

Components	**3 mL Endotoxin Removal Beads** or **5×3 mL Endotoxin Removal Beads**
Capacity	For up to 4.2×10^6 Endotoxin Units (EU) or for up to 7000 ng DNA per mL of Endotoxin Removal Beads.
Product format	One vial contains 3 mL Endotoxin Removal Beads at a concentration of 60 mg/mL in deionized water containing 0.05% sodium azide.
Storage	Store protected from light at 2 – 8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

Bacterial endotoxins (lipopolysaccharides, LPS) show strong biological effects in human beings, many animals, and cell cultures even at very low concentrations. Similarly, bacterial DNA can cause pathophysiological reactions *in vivo* and in cell cultures. Due to the high toxicity of bacterial endotoxins and DNA *in vivo* and *in vitro*, the removal of these molecules from protein preparations is of great importance for many applications.[1] However, common protein purification procedures are often not effective with regard to removal of bacterial endotoxins and DNA. Therefore, recombinant protein preparations are often contaminated with endotoxins[2] and DNA[3] originating from host cells.

1.2 Applications

Endotoxin Removal Beads have been developed for the quick removal of endotoxins and DNA from purified protein preparations. A polycationic ligand covalently bound to the bead surface shows very strong interactions with polyanionic molecules, such as endotoxins and DNA[4,5].
Endotoxin or DNA can be removed by 98% at initial concentrations ranging from 2000 EU/mL to 10,000 EU/mL or 2000 to 10,000 ng of DNA per mL, respectively.
Endotoxin Removal Beads are stable in the presence of many detergents, and within a wide range of pH values (2–10) and temperatures (4–37 °C).

1.3 Reagent and instrument requirements

- Buffer, for example, 0.1 M sodium phosphate buffer
- Polypropylene tubes
- 0.22 µm filters
- MACSmix™ Tube Rotator (# 130-090-753) for incubation of protein preparation with Endotoxin Removal Beads.
- MACSiMAG™ Separator (# 130-092-168) for endotoxin removal.

2. Protocol

2.1 Sample preparation

Efficient removal of polyanionic contaminants (endotoxins, DNA) from protein preparations requires optimal buffer conditions.
At pH values below the pI value of the target protein, the protein shows a positive net charge. As a consequence, the polyanionic contaminants are masked and an effective removal of endotoxins and DNA is not possible. On the other hand, a negative net charge of the target protein can lead to binding of the protein to the polycationic ligand and to a loss of protein. For optimal removal of endotoxin or DNA, the pH value of the buffer should be close to the pI value of the target protein .

▲ **Note:** The recovery of protein can be optimized by using 0.1 M sodium phosphate buffer. However, it is recommended to determine the protein recovery under the particular experimental condition since recovery may vary depending on the properties of the protein.

2.2 Equilibration of Endotoxin Removal Beads

▲ Use 15 mg (250 µL) of Endotoxin Removal Beads per 1 mL of protein solution. The beads must be equilibrated twice with the buffer used in the protein preparation.

▲ If the endotoxin contamination is high, it is possible to use a bead concentration above 15 mg/mL. The use of bead concentrations above 15 mg/mL may lead to a decrease in protein recovery.

▲ For 15 mL or smaller tubes a total of 150 mg (2.5 mL) and for 50 mL tubes a total of 300 mg (5 mL) of Endotoxin Removal Beads should not be exceeded.

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1. Transfer an appropriate amount of Endotoxin Removal Beads into an empty polypropylene tube.

   ▲ **Note:** Do not add Endotoxin Removal Beads directly to the protein solution.

2. Place tube with Endotoxin Removal Beads in the magnetic field of a MACSiMAG Separator. Use tube rack to insert tubes from 1.5 mL to 5 mL in size. For more details, see the MACSiMAG Separator data sheet.
3. Allow the Endotoxin Removal Beads to adhere to the wall of the tube for 4 minutes. Do not move the tube while separating.
4. Retaining the tube in the MACSiMAG Separator, carefully remove supernatant and discard it. Avoid contact to the beads adhered to the wall.
5. Add a volume of buffer to the Endotoxin Removal Beads that is equal to the volume of the protein solution to be processed.
6. Remove the tube from the MACSiMAG Separator and resuspend Endotoxin Removal Beads by vortexing vigorously.
7. Incubate for 5 minutes using the MACSmix Tube Rotator (12 rpm).
8. Repeat steps 2–7.
9. Repeat steps 2–4.
10. Proceed to adsorption of endotoxin and DNA to Endotoxin Removal Beads (2.3).

2.3 Adsorption of endotoxin and DNA to Endotoxin Removal Beads and separation

▲ Do not freeze protein solution before endotoxin removal. Formation of endotoxin micelles and vesicles is increased by freezing. Removal of endotoxin micelles and vesicles is more difficult than removal of monomers.

▲ When using sodium phosphate buffer, do not store protein solution for more than 24 hours before endotoxin removal to prevent enhanced formation of vesicles.

▲ When using Endotoxin Removal Bead concentrations above 15 mg/mL, the separation of beads in the MACSiMAG Separator may be less efficient. Therefore, transfer the supernatant after separation into a fresh tube and repeat the separation step.

▲ Only use pyrogen-free materials and work under sterile conditions after the adsorption process to avoid recontamination.

1. Add the protein solution to the equilibrated Endotoxin Removal Beads. Remove the tube from MACSiMAG Separator and resuspend Endotoxin Removal Beads by vortexing vigorously.
2. Incubate for 10 minutes using the MACSmix Tube Rotator (12 rpm).
3. Place tube with Endotoxin Removal Beads in the magnetic field of a MACSiMAG Separator for at least 4 minutes.

   ▲ **Note:** Endotoxin Removal Beads can adhere to the tube cap during incubation in the MACSmix Tube Rotator. For efficient bead separation, rinse the cap by turning the MACSiMAG Separator 3–4 times with tubes inside.

4. Transfer the protein solution into a fresh endotoxin-free tube. When using more than 150 mg of Endotoxin Removal Beads, repeat removal step as often as necessary.
5. (Optional) To remove residual Endotoxin Removal Beads, place tube with supernatant in the magnetic field of the MACSiMAG Separator and repeat step 3.
4. (Optional) Filtration of the protein solution using a 0.22 µm filter is recommended to remove remaining particles.
6. Discard Endotoxin Removal Beads.

3. Troubleshooting

Endotoxin removal is not satisfactory

Make sure that the pH value of the buffer is above the pI value of the target protein to avoid masking of endotoxins by the protein.
Extend incubation time to up to 40 minutes.
Increase bead concentration from 15 mg/mL to 25 mg/mL. If required, the bead concentration can be increased to up to 50 mg/mL.
Use buffer with lower ionic strength, for example, 50 mM Tris buffer.

Protein recovery is not satisfactory

Reduce bead concentration to 5 mg/mL.
Use buffer with higher ionic strength. Optimal protein recovery should be achieved with 100 mM sodium phosphate buffer; addition of NaCl is not recommended.
Decrease pH value to achieve positive net charge on the protein.

Protein solution is contaminated with beads after separation

Repeat separation in MACSiMAG Separator.
If using 15 or 50 mL tubes prolong separation time to 10 minutes.
Filter the protein solution using a 0.22 µm filter.
Use a larger tube for separation.

4. References

1. Issekutz A.C. (1983) Removal of gram-negative endotoxin from solutions by affinity chromatography. J. Immunol. Methods 61: 275–281.
2. Petsch, D. and Anspach, F. B. (2000) Endotoxin removal from protein solutions. J. Biotechnol. 76: 97–119.
3. Sakata, M. *et al.* (2005) Chromatographic removal of host cell DNA from cellular products using columns packed with cationic copolymer beads. Chromatographia 62: 465–470.
4. Mitzner, S. *et al.* (1993) Extracorporeal endotoxin removal by immobilized polyethylenimine. Artif. Organs 17: 775–781.
5. Anspach, F. B. (2001) Endotoxin removal by affinity sorbents. J. Biochem. Biophys. Methods 49: 665–681.

5. Appendix

5.1 Buffer recommendations

Sodium phosphate buffer is recommended for pH values from 5.8 to 8.0. For pH values from 2.2 to 5.6, citrate/Na_2HPO_4 buffer was successfully tested. For pH values from 8.6 to 10.0, 2-(N-cyclohexylamino)ethanesulfonic acid (CHES) buffer (≥0.1 M) is recommended.

140-002-519.03

5.2 Tables

Protein	Isoelectric point (pI)	Buffer	Protein concentration [mg mL^{-1}]	Endotoxin concentration [EU mL^{-1}]	Protein recovery [%]	Endotoxin removal [%]
BSA	4.7	0.1 M Na-phosphate, pH 7.0	0.8	6000	96.30	99.50
BSA	4.7	50 mM Tris, pH 7.0	0.8	16000	67.70	99.98
Lysozyme	11.2	0.1 M Na-phosphate, pH 7.0	1.2	100	88.33	90.00
Lysozyme	11.2	0.05 M CHES, pH 10.0	1.5	4200	54.96	98.75
Myoglobin	6.8	0.1 M Na-phosphate, pH 7.0	0.6	5000	91.67	99.20
Myoglobin	6.8	50 mM HEPES, pH 7.0	0.6	15000	91.67	99.99
TNF-α	7.7	0.1 M Na-phosphate, pH 7.0	0.1	17000	71.43	77.65
TNF-α	7.7	50 mM Tris, pH 8.0	0.1	20000	0.00	99.93
IL-1β	5.9	50 mM Tris, pH 8.0	0.2	1400	86.96	99.29
mIFN-α	9.1	Phosphate-buffered saline	0.2	400	70.00	75.00

Table 1: Endotoxin removal from various protein solutions. The Endotoxin Removal Bead concentration is 15 mg/mL.

Source of endotoxin	Endotoxin concentration [EU mL^{-1}]	Endotoxin removal [%]
Escherichia coli 0127:B8	10000	99.27
Escherichia coli 0111:B4	10000	98.64
Escherichia coli K-235	10000	99.27
Klebsiella pneumoniae	50000	99.19
Serratia marcescens	20000	96.70
Salmonella enterica, serotype typhimurium	1700	92.33

Table 2: Removal of endotoxins from various sources out of a 1.0 mg/mL BSA solution in 0.1 mol/L sodium-phosphate buffer. The Endotoxin Removal Bead concentration is 15 mg/mL. BSA recovery was above 84% at any rate.

Buffers/reagents	Acceptable concentration
Acetate buffer	0.1 mol L^{-1}
CAPS	0.05 mol L^{-1}
CHES	0.05 mol L^{-1}
Citrate/phosphate buffer	0.1 mol L^{-1}
Glycin/NaCl/HCl buffer	0.1 mol L^{-1}
HEPES	0.1 mol L^{-1}
MES	0.1 mol L^{-1}
MOPS	0.1 mol L^{-1}
Sodium phosphate buffer	0.1 mol L^{-1}
TRIS	0.1 mol L^{-1}
EDTA	50 mmol L^{-1}
Glutathione	10 mmol L^{-1}
Imidazole	250 mmol L^{-1}*
NaCl	0.1 mol L^{-1}*
Triton X-114	1%*
Tween 20	2%*
Urea	8 mol L^{-1}
* increasing concentration may lead to lower endotoxin removal efficiency	

Table 3: Tested substances which do not interfere with the endotoxin/DNA adsorption process.

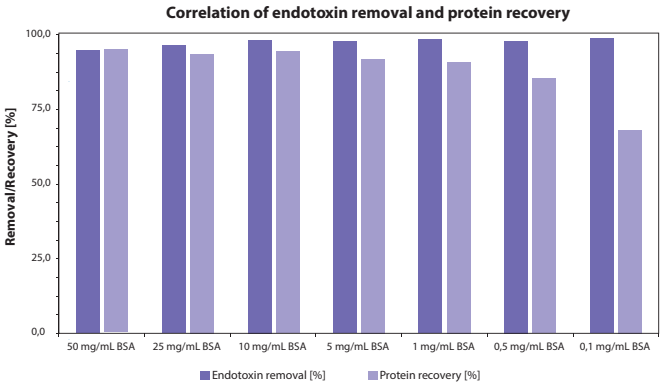

Figure 1: Correlation of endotoxin removal and protein recovery. The Endotoxin Removal Bead concentration is 15 mg per mL of 0.1 M Na_2PO_4 buffer. The incubation time is 10 minutes.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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