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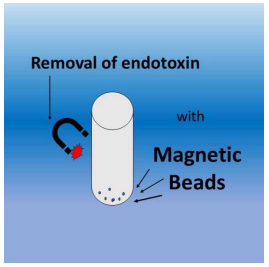
Quick and simple removal of endotoxins from purified protein solutions with magnetic beads



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Protocol status: Working
We use this protocol and it's working

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Abstract

Endotoxins (lipopolysaccharides) can affect the results of protein solutions in many ways, so it is important to eliminate them before in vitro and in vivo testing. There are several resin and chromatography- based solutions on the market. However, the use of these commercially available endotoxin elimination methods leads to significant losses of protein.

E. coli produces endotoxins as it is utilized to express a variety of commercially accessible recombinant proteins. The presence of trace amounts of endotoxins has negative effects on proteinsolutions. Therefore, we have developed magnetic beads that eliminate such endotoxins when introduced into solutions. There is no loss of proteins in these applications. Magnetic beads offer a simple and fast way to remove these endotoxins. They are also cost-effective because the beads used can be regenerated and reused multiple times.

Materials

Equipment needed

- Magnetic beads **(Genekam Endotoxin Removal Kit, SB0146)** sterile product
- Magnetic rack **(Genekam Magnetic separator for different tubes: 6 x 1,5 -2ml, SB0196)**
- Buffer solution (PBS)
- Pipettor
- Pipette tips
- Centrifuge optional
- Lamina flow, if user wants to have the whole process sterile

Safety warnings

-Keep the magnetic beads away from sun light.
-If the package and the bottles are damaged don´t use the kit.
-Read the material safety data sheet.

Precautions

-The kit is intended for in vitro use only.
-The kit should only be used by trained persons.
-The user must read the manual for use carefully.
-The kit should not be used after the expiry date.
-The user should work very cleanly during the removal process.
-Decontaminate the instruments regularly (once a week).
-The user should wear protective gloves and laboratory clothing.

Before start

-Read the protocol before start.
-Check the contents of the kit.
-The product (magnetic beads) is sterile.
-If the user want to have a sterile product, user should work under laminar flow.

Working protocol

44m

1 Performing of the isolation:
The user should use 10µl of magnetic beads for 250µl solution, from which the endotoxins are to be removed. If the user has 1 ml of solution, 40µl of magnetic beads should be used.

2 The user must first wash the specified quantity of magnetic beads in 1000µl buffer solution (PBS). For this purpose, 1000µl buffer solution is added to the beads, the tube is placed in the magnetic rack and the liquid is pipetted off after 2 minutes while the magnetic beads remain in the tube. Resuspend the beads in corresponding amount of buffer solution (40µl). 3m

3 This protocol is for 1 ml of the solution, from which user wants remove the endotoxins. Take 1 ml of the solution and add 40µl of the magnetic beads to this.

4 Leave the mixture at room temperature for 30 minutes in dark. Shake occasionally. 30m

5 Place the tube in the Genekam magnetic rack for 3 minutes. During this step, the magnetic beads are attracted towards the magnet. 3m

6 Remove the liquid with a pipette tip carefully without distrubing the magnetic pellet and collect it in a new tube. 3m

7 A test should be carried out to check whether the endotoxin has been extracted from the liquid. It is recommended.

8 Used magnetic beads can be regenerated and reused. 5m

9

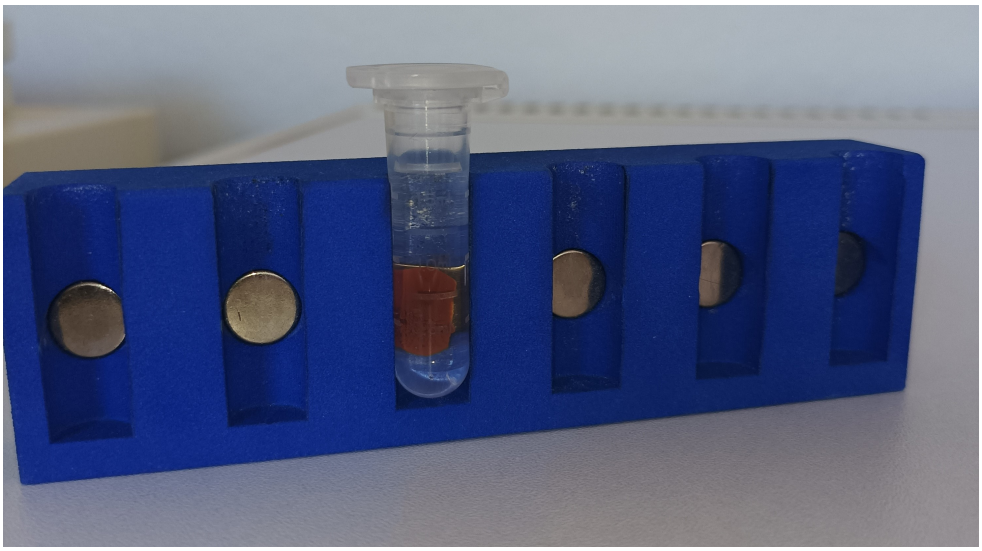

Figure 1: Tube with magnetic beads in the Genekam magnetic rack.