ProductInformation

POLYMYXIN B - AGAROSE
Sigma Prod. No. P1411

CAS NUMBER: N/A

PHYSICAL DESCRIPTION:

Matrix: Cross-linked 4% beaded agarose (activated by cyanogen bromide)
Attachment: Polymyxin B (Sigma product no. P1004) is attached through an amino group, with a 1-atom spacer
Ligand immobilized: Approximately 1 mg per mL
Binding capacity: 200-500 µg lipopolysaccharide from Escherichia coli serotype 0128:B12 per mL
Form: suspension in 50% glycerol containing 0.02% sodium azide

STORAGE / STABILITY AS SUPPLIED:

The resin should be stable at least one year stored at 2-8°C. Freezing the suspension will damage the agarose beads.

GENERAL REMARKS:

Polymyxin B is a strongly cationic cyclic antibiotic isolated from Bacillus polymyxa. It inhibits growth of microorganisms, including E. coli. It is a variable mixture of B1 and B2, with approximate molecular weight 1450. Polymyxins combine with cell membranes and disrupt normal permeability to small molecules.[1] "Polymyxin B and the other polymyxin antibiotics act primarily by binding membrane phospholipids and disrupting the cytoplasmic membrane...Polymyxin B has a bactericidal action on most Gram-negative bacilli, except Proteus spp....not active against Neisseria species, ... most fungi and Gram-positive bacteria."[2] General properties and applications have been reported[3-5], including its use in a bacterial permeabilization reagent.[6]

Usage of Polymyxin B Sulfate is often reported in terms of unit activity: "one unit is contained in 0.000119 mg of the second International Standard Preparation (1969) of Polymyxin B Sulfate which contains 8403 units per mg."[2]

Because of its affinity for bacterial cell membranes (which are lipopolysaccharides, also called endotoxins), Polymyxin B has been immobilized on agarose and used to remove endotoxins from solutions.[7,8] Histamine agarose has been used for the same purpose.[9,10]

P1411 is tested for its ability to bind LPS from E. coli serotype 0128:B12. A general protocol for use of polymyxin B-resins to remove endotoxins is given below.

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SUGGESTED PROTOCOL:[11]

1. The resin should be washed with 3-5 column volumes of buffer to remove the glycerol storage solution. (Endotoxin-free 0.1 M ammonium bicarbonate buffer pH 8.0 may be used.) Pack immobilized polymyxin B (10 mL gel) in a disposable polypropylene column.
2. Equilibrate the column with 100 mL buffer.
3. Dissolve sample, for example, bovine serum albumin (BSA, 400 mg, 80 E.U./mg) in 2.0 mL buffer, and apply to the column.
4. After the protein solution has completely entered the gel, elute with endotoxin-free buffer at pH 7.8, at a flow rate of approximately 0.2 ml/minute, while collecting 1.0-mL fractions.
5. Collect the fractions containing protein (BSA) and immediately lyophilize. Endotoxin testing of purified BSA should indicate a level of less than 1 E.U./mg protein. If the actual level is higher, recycle the protein through this resin to remove further quantities of endotoxin.

The polymyxin B agarose can be regenerated at least 10 times.[11] For regeneration, wash the column with 50 mL 1% sodium deoxycholate solution, followed by 50 mL endotoxin-free water. The resin can be stored in 25% ethanol at 4°C.

REFERENCES:

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