BESTCHROM
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MegaPoly AT Analytical antibody column

Instruction for use

Bestchrom (Shanghai) Biosciences Ltd.

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1. Product introduction

MegaPoly AT is analytical antibody column used for the HPLC system at high flow rate and high pressure. Its pre-packed resin uses porous polystyrene-divinylbenzene microspheres as its base matrix, which is coupled on alkali-resistant Protein A ligand after hydrophilic treatment on its surface. The resin can efficiently bind with IgG and Fc fusion protein, being applicable for the quantitative analysis and small-scale purification of antibodies and fusion proteins.

MegaPoly AT features:

- Monodisperse polystyrene-divinylbenzene microspheres made base matrix, better tolerance towards high pressure, higher flow rate.
- Hydrophilic treatment on surface, minimized non-specific adsorption, ensuring accuracy in testing results.
- Designed for high pressure column, suitable for HPLC operation.
- Alkali-resistant protein A ligand enables selective binding with IgG and Fc fusion protein, ensuring accuracy in testing results.
- Operation at 2mL/min normal flow rate and 4mL/min maximum flow rate, significant improvements in analysis efficiency.

2. Resin technical parameters

Physical appearance	White slurry, can be layered after placing
Base matrix	polystyrene-divinylbenzene microspheres
Average particle size	20μm
Functional groups	Alkali resistant Protein A
IgG binding capacity	~35mg/mL
Chemical stability	pH2-9：6M CH_6ClN_3、8M urea、20% ethanol、0.5M NaOH
pH stability	2~10(work)
Temperature tolerance	2~40℃，no freezing
Max pressure	20MPa
Storage	2~8℃，20% ethanol
Max flow rate	4mL/min

3. Column technical parameters

Particle size μm	Column volume mL	Inner diameter×bed height mm×mm	Column body material	Recommended flow rate+	Storage solution++
20	0.1	2.1×30	PEEK	2mL/min	20% ethanol

+ Recommended flow rate is used for operation, Minimum flow rate or 2mL/min flow rate is used for CIP and organic solution.

++ degas is necessary for 20% ethanol.

4. Instruction for use

4.1 Connect column to chromatography system

- Open the package and get the column.
- Check for column condition, contact your Bestchrom sales rep for any damage noticed.
- Turn on the chromatography system, make sure the system is degassed, set flow rate at 1mL/min.
- Connect to system：

  Connect the column to system according to the flow direction of column, normal operation should be in conformity with flow direction and reverse flow direction should be only used for contaminant rinsing.

4.2 Pre-treatment for column

- Rinsing：

  Column is preserved in 20%ethanol during transportation. rinse it with 2CV purified water.

4.3 Chromatography method

- Choose buffer：

  Binding buffer：20~50mM phosphate or Tris，0.1-0.2M NaCl or KCl，pH6.0~9.0.Binding is strong when pH is high, salt is used for reduction of impurities and non-specific adsorption.

  Elution buffer：25~100 mM phosphoric acid, glycine, citric acid, acetic acid，pH 2.0~3.5.
- Flow rate：max flow rate is 4mL/min.
- Sample preparation：To avoid column blocking with sample, adjust the sample pH and conductivity rate to the same level of buffer . Samples need to be filtered with a 0.45 μm microporous membrane prior to loading.
- Washing：Wash the column with binding buffer till the outlet UV absorption is close to base line.
- Elution：Elution buffer consist of HCl, glycine, citric acid, acetic acid and other low pH components. Since binding and elution of antibody varies from various proteins, best elution condition should depend on the experiment condition.
- Re-equilibration：Rinse with binding buffer till pH and conductivity rate reach the same level of buffer. Load sample for the second time and repeat the step.

5. Clean in place(CIP)

Contaminants can gradually accumulate in the column as use of resin increases. Regular CIP can effectively prevent the accumulation of contaminants and therefore keep the column function normally. Determine the frequency of CIP according to the degree of contamination of the resin (if the contamination is serious, CIP should be carried out after each use to ensure repeatability of the results).

Wash resolutions can be used for MegaPoly AT column are: 0.1M NaOH，2~6 M guanidine hydrochloride，1 M acetic acid，20% ethanol，1 M acetic acid+ 20% ethanol，20% isopropanol.

6. Sterilization

MegaPoly AT column can be treated with 70% ethanol for more than 12h to reduce risk of microbial contamination.

7. Storage

MegaPoly AT Column storage condition：

Fill the column with 20% ethanol, refrigerate at 2-8℃. Column outlets should be blocked with plugs to prevent dry-up.

8. Cautions

- MegaPoly AT column body is made of PEEK. Screwing should be completed manually. Metal spanner should be avoided to prevent scratch.
- All samples and buffers should be filtered with 0.2μm or 0.45μm membrane to prevent column plugging.
- Avoid dramatic temperate volatility in buffers and columns for better separation effect.
- Avoid exposure to direct sunlight.
- Column can be used in fridge provided lower flow rate is used.
- Reverse rinsing can be used for serious clogging issue with flow rate being reduced below 50% of its normal flow rate.
- Column is usually operated under high pressure, buffer and sample leakage are possible when column is not tightened completely. Protective gloves are necessary for handling a column with leakage.
- Due to the small size of microspheres in column resin, a mask is necessary when using column to prevent inhale of microspheres.
- In case when polymer microspheres accidentally splashed into eyes, pull on eyelids to flush immediately with plenty of water. Avoid touching eyes with hands.

9. Order information

Column	Column volume	Material	Item code	Pack size
MegaPoly AT	0.1mL	PEEK	AC002	1 pcs