ION EXCHANGE INSTRUCTIONS

PROCEDURE FOR USE

IEX RAPID RUN™ Agarose Beads

DESCRIPTION

IEX Rapid Run™ Agarose Beads are designed to purify a wide range of biomolecules according to their difference in surface charge such as:

- Proteins
- Peptides
- Nucleic Acids

The agarose resin matrix is highly crosslinked offering many advantages:

- High chemical stability which assures that characteristics such as capacity, elution behavior, pressure and flow velocity are unaffected by the solutions that are commonly used in process chromatography and cleaning procedures
- High physical stabilities that demonstrate good flow characteristics and low back pressure
- High rigidity of the matrix minimizes volume variations during change of pH or ionic strength
- Optimal porosity result in high dynamic binding capacity
- Reliable and consistent performance
- Readily scalable

Q, DEAE and CM Rapid Run™ Agarose Beads are supplied as a suspension in 20% ethanol; SP Rapid Run™ Agarose Beads as a suspension in 20% ethanol and 0.2M sodium acetate.

INSTRUCTIONS

Removal of preservative

Wash the resin 5 times with 5ml of deionized water/ml resin to remove the preservative using a vacuum liter and spatula to stir gently between additions.

Column packing

1. Prepare a 50% slurry concentration adding water to the washed resin
2. Gently stir resin to homogenize the slurry suspension. Do not use a magnetic stirrer as it can damage the resin.
3. Place a funnel into the top of the column and slowly pour the slurry down the column to avoid the formation of bubbles. It is preferable to add the entire volume of suspension in one step and avoid repeated additions.
4. Repeat previous step until the required column height is obtained. In smaller columns (1-20ml) repeated additions might still be needed.
5. Gently insert the adapter into the top of the column and make sure no air bubbles are trapped above the resin surface.

6. Connect the pump to the column at the packing flow rate and finally check that the column bed height remains constant. The packing and operational flow rates cannot exceed the maximum pressure of the column.
7. Equilibrate the column with the buffer solution until the pH and conductivity are constant.

Evaluation of packing quality

To check the column packing an efficiency test should be run to determine the theoretical plate number and peak asymmetry factor.The most common procedure is to run a pulse test.
The column is tested by pumping acetone (1-2% in water) using a sample volume of corresponding to 1% of total column volume.
The recommended operational flow velocity for 90µm beads is 20cm/h

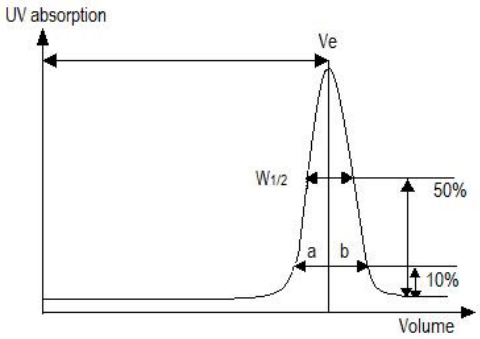

Figure 1. Typical chromatogram from a pulse test using acetone

The number of theoretical plates (N) is calculated using the formula:

$$N = 5.54 \times (V_e/W_{1/2})^2$$

V_e = Peak retention or elution volume
$W_{1/2}$ = Peak width at half peak height
(V_e and $W_{1/2}$ should be in same units)

Quality of packed bed can also be expressed by reduced plate height (h) independent of particle size:

$$h = HETP/d_{50V}$$

HETP = L/N = Height equivalent of a theoretical plate

L= Bed height
d_{50V} = Mean particle size
(L and d_{50V} should be in same units)

For preparative applications a reduced plate height of ≤ 3 is acceptable.

Peak asymmetry factor (As) is calculated using the formula:

$$As = b/a$$

a = First half peak width at 10% peak height
b = Second half peak width at 10% peak height

A typical acceptable range could be 0.8<As<1.8

Regeneration

The best way to regenerate the resin after each cycle is to remove any unwanted bound material by elution with a high ionic strength solution (1-2M NaCl) at low pH (for DEAE and Q) and at high pH (for CM and SP) followed by washing with regeneration buffer until the column reach constant conductivity and pH values.

Cleaning in place (CIP) and sanitization

Cleaning in Place (CIP) should be run to remove any bound precipitates, lipids, denatured proteins, viruses and other unknown contaminants present after regeneration. If not removed, these can affect resin and column performance as well as carry over to the next cycle. Therefore one CIP cycle is recommended at least every 5 cycles operation depending on sample complexity.

To remove precipitated bound proteins or lipoproteins, wash the column with 4 to 10 bed volumes of up to 70% ethanol or 30% isopropanol. Alternatively, wash the column using 0.5% non-ionic detergent (in 1.0 M acetic acid). Residual detergent is removed by washing with 5 bed volumes of 70% ethanol.

Sanitization using NaOH to reduce microbial contamination of the resin bed to acceptable levels without dismantling the column is recommended. A concentration of 0.5 to 1 M NaOH with a contact time of 1 hour has proved effective for most microbial contamination. Spore forming bacteria require more complex CIP solutions and longer contact time for efficient sanitization.

Sterilization

The resins can be autoclaved in their salt form (Cl^- for DEAE and Q, Na^+ for CM and SP) at pH=7 and 121ºC for 30 minutes for sterilization.

Storage

Store SP Rapid Run™ Agarose Beads in 20% ethanol containing 0.2 M sodium acetate at 4 - 30°C.
Store the CM, DEAE and Q Rapid Run™ Agarose Beads in 20% ethanol at 4 - 30°C.