NUCLEOGEN® DEAE

The NUCLEOGEN® family offers remarkable performance for the separation of biopolymers. It is a series of silica gel based anion exchangers available with pore size of 60 Å, 500 Å, 4000 Å. The chemistry of the surface coating was elaborated for present day separation problems in nucleic acid research. The favourable interplay of these qualities is seen best from the separation of oligonucleotides, of high molecular weight RNAs and of plasmids. The small particle size together with the monomolecular, hydrophilic coating result in a very high resolution and a manifold reduction of chromatographic separation times combined with a high degree of recovery. The stable microparticles show neither swelling with salt of pH gradients nor a break-down of the column packing even with high flow rates and high pressure up to 300 bar (4200 psi). Reequilibration to starting conditions is reduced to several minutes.

Operating conditions for NUCLEOGEN® columns

Mobile phase

The NUCLEOGEN® columns can be used with aqueous buffers in the pH range 2.5 – 8.0, with denaturating agents (6 M urea, 70% formamide), non-ionic detergents and most organic solvents.

However, the eluent composition should meet the following requirements:

- Only buffers with a pH between 2.5 and 8.0 should be used.
- For elution salts like LiCl, NaCl, KCl, $(NH_4)_2SO_4$ and sodium acetate are recommended.
- Denaturants such as 8 M urea, 8 M guanidinium-KCl, 60% formamide, neutral detergents, ethylene glycol or similar substances as well as lower alcohols and acetonitrile can be used.
- Eluents with reactive groups (e. g. ketones, aldehydes) should not be used, because they can modify the functional groups causing a loss of separation efficiency.
- For nucleic acids it is of advantage to work with aqueous buffers in the presence of 4 – 7 M urea, 30 – 60% formamide, 10 – 30% acetonitrile to achieve optimum resolution for high chain lengths as well. Formamide p. A. is used without prior purification, and the absorption in this case is measured at 270 nm.
- Prior to use all eluents should be filtered through a 1 μm filter to avoid blocking of the column by undissolved particles from the buffer.
- To avoid formation of bubbles in the column or detector, all eluents have to be degassed prior to use. The buffer can be degassed in vacuum (5 min, glass filter pump, simultaneous stirring with a magnetic stirrer), by ultrasonic treatment (3 – 5 min) or by flushing with helium.

Preparation of samples

- To avoid precipitation of substances in the column dissolve your sample in the starting buffer or – if the sample is dissolved in another low salt buffer – adjust to chromatographic conditions by adding concentrated buffer solution.
- Remove undisssolved components in your sample by filtration through a 0.2 μm filter or by a short centrifugation to avoid blocking of the column.
- For the separation of nucleic acids it is important to incubate the samples at 56 °C for 5 minutes to transfer all nucleic acids to a uniform conformation.

Column installation

- For delivery the column is conditioned with 100% methanol. Methanol is not completely miscible with salt containing buffers. To avoid precipitation of salts, the methanol has to be washed out with distilled water. After 2 – 3 column volumes of water you can proceed to the buffer system desired.
- Please note the flow direction of the column. Optimum resolution is only achieved in the flow direction indicated for the column.
- Before connecting the column to the HPLC instrument check for unhindered flow of the mobile phase (water) through the capillaries and detector. Flush the system with degassed water to remove air bubbles.
- Remove the end caps from the column and connect the column inlet to the filled injection valve and the column exit to the detector.
- Start with a flow rate of 0.5 mL/min and increase slowly to the flow rate desired. Make sure the flow is unhindered, i. e. the back pressure does not deviate more than 15% from the value indicated in the test chromatogram. The back pressure should never exceed a maximum of 250 bar.
- Flush the column with 2 – 3 times its volume of water.

Column operation

- Avoid fast changes in flow, i. e. abrupt pressure changes in the column. Pressure waves with high pressure peaks may cause destruction of the column packing.
- Increase the flow rate in small steps until the desired flow rate is reached.
- After changing to the elution buffers wash the column with the salt buffer until again a stable detector signal is obtained.
- Avoid abrupt changes in ionic strength and viscosity. After finishing a separation the high ionic strength should be decreased to starting conditions with lower ionic strength over a short gradient (e.g. 100% to 0% in 10 min, 1 mL/min)
- Equilibrate the column with the starting buffer.
- To remove impurities from the column, inject several sample of 100 μL 2 M KCl (or NaCl, LiCl, $(NH_4)_2SO_4$) in 6 M urea, 0.02 M K-Phosphate, pH 6.8 and equilibrate to starting conditions.
- For chromatography the temperature should not be subject for major fluctuation. If elevated temperatures have to be used, for protection of the column please note the following:
  a) The temperature should not be raised above 70 °C.
  b) All eluents have to be thoroughly degassed.
  c) Increase the temperature only while the flow is constant.
  d) After separation is completed decrease the temperature to ambient while the flow is constant.

Cleaning of column

Impurities on a column which are noted by a decrease in separation efficiency or an increase in back pressure may in most cases be removed by the following procedure:

- Wash the column with 10 column volumes of water
- Wash the column with 10 column volumes of 2 M KCl, 6 M urea, 0.01 M K-phosphate, pH 6.8
- Wash again with 10 column volumes of water.
- Wash the column with 10 column volumes of methanol.
- Wash the column with 5 column volumes of water and equilibrate the column to your chromatographic conditions.

Storage of columns

The column should never – not even over night – be subjected to unnecessarily high salt concentrations or pH values above pH 7. Especially halides like Cl^-, Br^- lead to corrosion of the column and thus to its destruction. Wash the column with bidistilled water (pH about 6.0) until all salts have been washed out (about 4 – 5 column volumes). To prevent growth of bacteria 0.01 % sodium azide may be added to the water. If a column is not to be used for a longer period of time (several days) it should be equilibrated with methanol and stored in methanol. Wash the column with 4 – 5 times its volume of distilled water and subsequently equilibrate with 4 – 5 times its volume of methanol.

Store the columns well closed at room temperature.

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