GE Healthcare

illustra™
Sephacryl S-1000 Superfine

For the purification of DNA up to 20 000 base pairs, dextrans up to 10^8 molecular weight, and spherical particles up to 400 nm.

Product booklet

Code: 17-0476-01 (750 ml)

Page finder

1. Legal

Product use restriction
Sephacryl™ S-1000 Superfine medium has been designed, developed and sold **for research purposes only**. It is suitable **for *in vitro* use only**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).
It is the responsibility of the user to verify the use of Sephacryl S-1000 Superfine medium for a specific application range as the performance characteristics of this product has not been verified to a specific organism.

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2. Handling

2.1. Safety warnings and precautions

Warning: *For research use only*.
Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

We recommend that this product and components are handled only by those persons who have been trained in laboratory techniques and that they are used in accordance with the principles of good laboratory practice. As all chemicals should be considered as potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.
See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store at ambient temperature (4°C to 30°C). **Do not freeze**.

2.3. Expiry

For expiry date, please refer to outer packaging label.

3. Components

3.1. Contents

Cat. No.	17-0476-01
Sephacryl S-1000 Superfine medium (Sephacryl is supplied as a suspension in distilled water containing 20% ethanol as a preservative)	750 ml

3.2. Materials to be supplied by user

Eluent buffer (refer to section 5.1 for details)

3.3. Equipment to be supplied by user

Glass rod for mixing the medium
Peristaltic pump
A suitable column
Flow adapter
Extension reservoir

The following equipment can be purchased from GE Healthcare

Item	Catalogue number
C 26/70 column	19-5202-01
AC26 Adapter	19-5207-01
LV-3 manual valve	19-0016-01
RC 26 column packing reservoir	19-5208-01

4. Description

4.1. The basic principle

Sephacryl S-1000 Superfine allows preparation of DNA and separation of very large polysaccharides, proteoglycans and small particles (e.g., membrane-bound vesicles and viruses) by the process of gel filtration. Molecules larger than the largest pores in the matrix are excluded from the matrix and elute first. Intermediate size molecules penetrate the matrix to varying extents, depending on their size. Penetration of the matrix retards progress through the column; very small molecules elute last. The volume required to elute these small molecules is dependent on the volume available both inside and outside the pores (i.e. the bed volume).

Sephacryl S-1000 Superfine is optimized for the purification of DNA up to 20 000 base pairs, including plasmids, vesicles and viruses; dextrans up to 10^8 molecular weight; and spherical particles up to 400 nm.

4.2. Product specifications

Format	**Gravity flow column**
Principle	Gel filtration
Matrix	Spherical allyl dextran and N, N'-methylenebisacrylamide
Column buffer	Distilled water containing 20% ethanol as a preservative
Fractionation range for dextrans (Mr)	500 000 – 100 000 000
Exclusion limit for DNA	20 000 base pairs

Particle size range	40 – 105 µm
pH[1] stability (working and long term)	3 - 11
pH[1] stability (short term)	2 – 13
Maximum[2] operating flow rate (approx.)	40 cm/h[3]

1. pH stability long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance. pH stability short term refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures. All ranges given are estimates based on our knowledge and experience.

2. At room temperature in aqueous buffer. The flow rate giving optimal resolution depends on the sample.

3. Flow rate is calculated from measurement in packed columns with an i.d. (internal diameter) of 2.6 cm. A column height of 60 cm is used for Sephacryl.

5. Protocol

5.1. Important considerations before starting

Choice of eluent

- Eluent composition does not directly influence resolution
- To avoid ionic interactions, the ionic strength of the eluent should be at least 0.15 M
- Choose an eluent providing good solubility and stability for the sample
- The eluent can often be chosen to simplify a later separation stage e.g. the column can be equilibrated and eluted with the start buffer for a subsequent ion exchange separation

Eluent preparation

- Use distilled water
- Use HPLC or analytical grade solvents, salts and buffers
- Make and store the eluents in clean glassware
- Filter the eluents through a 0.22 µm sterile filter and degas before use
- Store the eluents at 4°C when not in use. To prevent bacterial growth an antimicrobial agent can be added
- Equilibrate the eluents to ambient temperature before use to prevent formation of air bubbles (it is recommended to degas before use if the eluent has been in storage)

Sample preparation

- Use a sample volume of 0.5% to 4% of the column volume.
- In group separations (desalting) the sample volumes can be up to 30% of the column volume

- When repeating runs prepare the sample using the same method and keep the sample concentration and volumes constant each time
- Choose chromatographic conditions under which the sample is stable and soluble
- Centrifuge (e.g. 10 000 g for 10 min) or filter sample to remove microparticles (be sure to select a solvent resistant filter if samples are dissolved in organic solvents)
- If the sample has high viscosity, dilute it with the eluent (avoid >30 mg protein/ml sample)
- Never apply a turbid solution to the column; turbidity indicates sample insolubility which may be due to incorrect ionic strength or pH
- Store samples in the cold unless this leads to precipitation; avoid long storage unless you can store at –70°C
- Use distilled water
- Use HPLC or analytical grade solvents, salts and buffers

Sample application

- Make sure the sample is recently filtered or centrifuged before applying it to the column
- A prefilter between injector and column is not recommended unless automated injections are performed (a prefilter reduces the resolution)

Optimization

Parameters to change are:

1. Sample volume (a smaller sample volume gives better resolution)
2. Flow rate (a lower flow rate gives better resolution for high molecular weight components (proteins), but the opposite may be true for small components (small peptides) since they diffuse more

quickly, and the longer the separation time, the wider the sample zones become)

3. Column length (the resolution of two separated zones increases by the square root of the column length); the effective bed height can be increased by coupling two columns in series. Use the Union 1/16" male/1/16" male, Code No. 18-1120-93 to couple columns in series

Buffer choice and pH are normally of minor importance; however, low pH may enhance hydrophobic interactions and can be used to improve separation in some cases, e.g. peptides (mixed-mode separation)

5.2. Column packing procedure

1. Mount the column vertically and fill with eluent buffer. This is required to wet the walls of the column and remove air from the bed support.

Note: this procedure is also performed to ensure there are no leaks in the column.

Note: refer to section 5.1 for advice on eluent buffer preparation.

2. Drain the eluent buffer, leaving about 1 cm remaining in the column.

3. Open the bottle of Sephacryl S-1000 Superfine and thoroughly mix the contents using a glass rod, DO NOT use a magnetic stirrer.

4. Determine the packed bed volume required and remove ~150% of this volume from the bottle of mixed Sephacryl S-1000 Superfine.

5. Once the desired volume has been removed, allow the gel to settle and decant the excess distilled water.

Note: DO NOT at any time allow the gel to become dry. Sephacryl S-1000 Superfine is supplied as a suspension in distilled water containing 20% ethanol.

6. Resuspend the Sephacryl S-1000 Superfine in eluent buffer to make a slurry containing ~70% settled gel.

Note: the slurry should not be so thick as to retain air bubbles.

7. Degas the Sephacryl S-1000 Superfine slurry under vacuum.

8. Carefully pour the slurry into the column (use an extension reservoir if necessary). See Figure 5.2.1 on page 13 for details of apparatus set up.

Note: the gel should be packed rapidly.

9. Fill the column (or extension reservoir) with buffer – taking care not to disturb the gel. Connect a peristaltic pump and pack at the following rates (see Table 1 below).

Note: it may be more convenient to first allow the gel to sediment into the column during flow under a hydrostatic pressure of about 100 cm of water, and then connect the pump to the column to continue packing.
If no pump is available, the hydrostatic pressure should be adjusted to achieve the same flow rates as indicated in table 5.2.2.

Table 5.2.2. showing flow rates for packing with a peristaltic pump.

	Column diameter	
	<2.6 cm	**5 cm (K 50)**
S-1000	40 cm/h	30 cm/h

(To convert linear flow rate (cm/h) to volume flow rate (ml/h) multiply by the cross-sectional area of the column).

Note: these flow rates apply for beds up to ~50 cm in height. For longer beds, decrease the packing flow rate, calculated using the equation below:

$$\text{Packing flow rate} = \frac{50}{\text{Gel bed length (cm)}} \times A$$

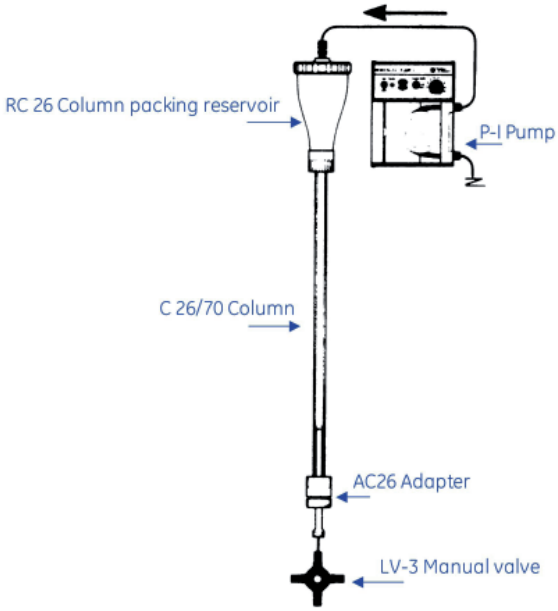
Where A is the flow rate for the gel and column in question given in Table 5.2.2. above.

10. After two bed volumes of eluent buffer have passed through the column, remove the gel reservoir and insert a flow adapter so that it just touches the upper gel surface.

11. Following insertion of the flow adapter, pack the column for an additional two bed volumes at the same flow rate.

Note: some further settling of the gel may occur with extended use, in which case the adapter can simply be readjusted.

Figure 5.2.1 showing apparatus set up



5.3. Cleaning protocols

Cleaning protocols

Three cleaning protocols are recommended, these are as follows:

A. Simple: for regular cleaning

B. Rigorous: for when the column is contaminated

C. Harsh: to be used as a last resort

A. Simple cleaning

- The packing effciency is crucial; to avoid losing any performance, only clean the column when the contamination causes an increase in backpressure:

1. Wash with 1/5 column volume (CV) 0.1 M NaOH
2. Wash with 1/5 CV 1 M acetic acid
3. Equilibrate with eluent until the baseline is stable

B. Rigorous cleaning

Clean the column if you observe any of the following:

- An increase in backpressure

1. Before cleaning, make sure that the high backpressure in the system is in fact caused by the column. Disconnect one piece of equipment at a time (starting at the fraction collector). With the pumps working, check the pressure reading after each piece is disconnected to determine the source of the backpressure (you will often find that a dirty prefilter causes the increase in backpressure).
2. Check the backpressure at the same stage during each run since the backpressure can vary within one run, e.g. injecting a sample and mixing different eluents may cause an increase in backpressure.

- A space visible between the adapter and filter
- A color change at the top of the column

• A loss of resolution

The following steps should be performed in sequence (NEVER exceed the column pressure limits):

1. Change the filter at the top of the column (Tricorn and HiLoad columns; see instructions for individual columns or filter kits). Since the contaminants are introduced with the liquid flow, many of them are trapped in the filter
2. Set the pressure limit control to the pressure limit given in the column instructions
3. Wash with 1 column volume (CV) 1 M acetic acid
4. Wash with 1 CV water
5. Wash with 1 CV 20% ethanol (run at a low flow rate)
6. Wash with 1 CV 0.1 M NaOH
7. Rinse with 1 CV water and a few injections of 1 M acetic acid
8. Equilibrate with buffer until the baseline is stable

Cleaning volume of 1 CV is only a guideline, the practical requirements are best determined by monitoring the baseline, which should be stable at the end of each step

C. Harsh cleaning

1. Chemical operations

- When planning a recovery operation, always take into account what caused the problem in the first place; various alternatives are given for each type of contaminant. Choose the most convenient according to the reagents you have available: if this does not work, try another.
- Do not exceed pressure limits given in the column instructions

Hydrophilic proteins and peptides:

- Wash the column (overnight, at low flow rate 15–30 cm/h) with

the solution which previously dissolved the material during sample preparation for example, an extraction solution or detergent.
- Wash the column overnight in 1 M acetic acid at 15–30 cm/h
- Fill the column with 1 mg/ml pepsin in 0.1 M acetic acid and 0.5 M NaCl and incubate overnight at room temperature, or 1 h at 37°C. After enzymatic digestion, thoroughly rinse the column with equilibration buffer.

Hydrophobic proteins and peptides:
- These are usually soluble in polar organic solvents such as 90% ethanol, 30% acetonitrile, or 30% isopropanol.
- If the percentage of organic solvent which best dissolves the contaminant is known, run this overnight at a low flow rate.

Nucleic acids:
- General: RNA and DNA are very soluble in solutions of low ionic strength: wash with 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 at low flow rate for 24 h at room temperature to dissolve precipitated nucleic acids.

RNA:
Inject 0.1–2 M NaOH (see Instruction for NaOH stability) and leave for 1 h, rinse with water, inject 0.1 CV of ribonuclease I solution (1 mg/ml in 0.1 M NaCl, 50 mM Tris-HCl, pH 7.5), incubate the column for 2 h at 37°C, rinse with at least 2 CV of 10 mM Tris-HCl, 1 mM EDTA, pH 8.0

DNA:
Add 0.1 CV Deoxyribonuclease I solution (1 mg/ml in 0.1 M NaCl, 10 mM MgCl2, 50 mM Tris-HCl, pH 7.5) to the column, incubate the column for 2 h at 37°C, rinse with at least 2 CVs of 10 mM Tris-HCl, 1 mM EDTA, pH 8.0. After enzymatic digestion, thoroughly rinse the column with equilibration buffer to remove trace amounts of

enzyme remaining in the system; special caution is recommended if subsequent separations of RNA or DNA are planned

Lipids:
- Wash the column overnight at a flow rate of 15–30 cm/h with detergent such as 0.2–1% Berol™ 185 in a basic or acidic solution. Remove detergent by washing with methanol, ethanol or isopropanol

Carbohydrates:
- Wash with 1 column volume of 0.1 M sodium tetraborate titrated to pH 8 with HCl. Note that sodium tetraborate precipitates with some metal ions such as Cu^{2+}

2. Mechanical operations
- Change the bottom filter - note that this may reduce the column efficiency
- Remove the top 2–3 mm of medium and discard
- Always check the efficiency of the column after mechanical cleaning

5.4. Storage of unused medium
- 4°C to 30°C
- Make sure the unused medium is protected against bacterial growth by storing in 20% ethanol or buffer containing an antimicrobial agent.

5.5. Storage of column
- 4°C to 30°C

Short term storage (e.g. overnight)

- Store the column connected to the system; a low flow rate through the column will prevent bacterial growth
- Store in the eluent used in separation

Long term storage

The following steps should be performed in sequence (NEVER exceed the column pressure limits):

1. Clean the column according to "Simple cleaning" (section 5.3)
2. Rinse the column and the system thoroughly with water to remove salt
3. Equilibrate the column with 2–3 column volumes of 20% ethanol: start the equilibration at a low flow rate and check the backpressure while equilibrating the column (mixing water and ethanol increases the backpressure)
4. Disconnect the column from the system
5. Seal the column inlet and outlet

5.6. Antimicrobial treatment

Prepacked columns: Sanitize

Bulk media: Sanitize/autoclave

Sanitization is the inactivation of microbial populations. When a packed column is washed with a sanitizing agent, the risk of contaminating the purified product with viable microorganisms is reduced. The most commonly used sanitization method in chromatography is to wash the column with NaOH. NaOH has a very good sanitizing effect and also has the additional advantage of cleaning the column; See instructions in section 5.3.b.

Bulk medium may be autoclaved in a wet format at pH 7 for up to 30 minutes at 120°C

6. Appendix

6.1. Related products available from GE Healthcare

Application	Product	Product Code	Pack Size
PCR and **enzymatic DNA reaction purification** (enzyme removal, buffer exchange, desalt, primer removal), 100 bp–10 kb size range Extraction of DNA from agarose gels	GFX™ PCR and Gel Band Extraction Kit	28903470	100 columns
Purification of oligonucleotides following synthesis (buffer exchange and de-salt). Gravity format, 500 µl loading volume	NAP™-25 columns	17-0852-01	20 columns
Unincorporated labeled nucleotide removal from a DNA labeling reaction Gravity flow	NICK™ columns	17-0855-02	50 columns
Gel filtration media Preparative / Macro fractionation (Mr 1000 – 5 000 000)	Sephacryl S-200 HR	17-0845-01	750 ml
	Sephacryl S-300 HR	17-0599-01	750 ml
	Sephacryl S-400 HR	17-0609-01	750 ml
	Sephacryl S-500 HR	17-0613-01	750 ml

Application	Product	Product Code	Pack Size
Gel filtration media			
Desalting and buffer exchange	Sephadex™ G-25 DNA grade	17-0572-02	100 g
Removal of dye terminators and labeled nucleotides.	Sephadex G-50 DNA grade	17-0573-02	100 g
	Sephadex G-100 DNA grade	17-0574-02	100 g

A full range of reagents can be found in the GE Healthcare products for life sciences catalogue.

If you need any further information, GE Healthcare technical services are always happy to assist.
http://www.gehealthcare.com/nap

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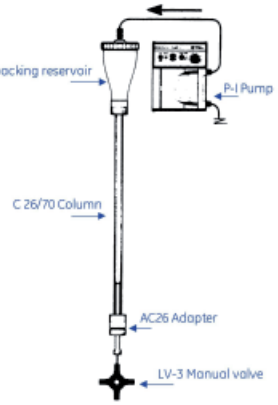
illustra™
Sephacryl S-1000 Superfine
Experienced user protocol 17-0476-01

For the purification of DNA up to 20 000 base pairs, dextrans up to 10^8 molecular weight, and spherical particles up to 400 nm

1. Mount column and fill with eluent buffer
2. Mix Sephacryl using a glass rod
3. Measure out ~150% of the desired packed bed volume
4. Decant excess distilled water
5. Resuspend Sephacryl in eluent buffer (70/30, Sephacryl/eluent buffer, v/v)
6. Degas the slurry
7. Pour slurry into column
8. Quickly pack column
9. Pass two bed volumes of eluent buffer through column
10. Insert flow adapter
11. Pass two bed volumes of eluent buffer through column



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