

Sephadex G-25 DNA Grade (SF)

Quick-start user guide

Product description

Sephadex™ G-25 DNA Grade superfine (SF) chromatography resin for purification of DNA fragments greater than 10 bases in length from small molecules such as salts by size exclusion. DNA fragments above 10 bases will be excluded from the resin and will pass through without retention compared to smaller molecules. The media is provided in bulk (dry) format to allow researchers to prepare their own columns for purifying nucleic acids.

The product is tested to ensure reproducibility and high recovery of DNA (> 90%) and is highly suitable for the purification of oligonucleotides or small DNA fragments following synthesis or in labelling applications.

Product	Pack size	Code number
Sephadex G-25 DNA Grade (SF)	100 g	17057202

Product specifications

Particle size dry	20–80 µm
pH stability	2–13
Fractionation range (Mr) (globular proteins)	1000–5000
Fractionation (Mp) dextrans	100–5000
Fractionation range (Mr) DNA exclusion limit	10
Storage	0.01 M NaOH

Instructions

Sephadex powder has to be hydrated before use. The resin must be fully swollen before packing into columns.

Swelling time: 72 hours at 20°C or 5 hours at 90°C

Step 1

Weigh out the appropriate amount of dry gel for the required bed volume (approx. bed volume: 4–6 mL/g dry gel).

Step 2

Add enough buffer to equal the total volume of the column plus 30% more.

Step 3

Stir. Excessive stirring should be avoided as it may break the beads: DO NOT USE MAGNETIC STIRRERS. The process is accelerated by using a boiling water bath, which also serves to deaerate the buffer.

Step 4

Decant the supernatant (after complete swelling) to remove fines.

Step 5

Reintroduce buffer to make a suspension; this should be fairly thick (about 75% settled gel).

Step 6

If degassing is required, use of an appropriate glass vessel attached to a vacuum line is recommended.

Once prepared, pour into an appropriate column. To equilibrate, pass 2 to 3 column volumes of the aqueous buffer to be used in the separation.

Swollen resin can be stored refrigerated at 2°C–8°C. for up to 1 month. For longer term storage, a bacteriostatic agent should be included in the buffer such as 20% ethanol or Kathon.

Elution buffers

Eluent composition should not directly influence the resolution that can be obtained:

1. For substances carrying charged groups an eluent containing a buffer e.g. Tris-HCl, sodium phosphate is used to control pH, and an ionic strength of at least 0.02 is recommended to safeguard against possible ionic interactions with the gel matrix. NaCl can be used for this purpose.
2. For product that is to be lyophilized: use volatile buffers e.g. ammonium acetate, ammonium bicarbonate or ethylenediamine acetate.

Shipping and storage

Ship at room temperature. Store at room temperature.

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