Technical Bulletin

Sephadex® G-25

BioReagent, for molecular biology, DNA grade, Superfine

S5772

Product Description

Sephadex® G-25, Superfine is a gel filtration chromatography product for desalting and buffer exchange of very large molecules. Sephadex® is prepared by crosslinking dextran with epichlorohydrin. Sephadex® products differ in their degree of cross-linking, and thus in their degree of swelling and their molecular fractionation range. On the general term "Sephadex" and other aspects of Sephadex® products:

- "Se" refers to "separation", and "dex" to dextran.[1]
- "G" refers to "Gel".[1]
- The G-number in a given Sephadex® listing refers to the water regain of the gel multiplied by 10, where water regain is defined as the maximum amount of grams of water taken up by 1 g of "dry xerogel".[1]

The designation "Superfine" indicates a smaller particle size which allows for shorter diffusion distances, highly efficient separations at high flow rates, and minimal non-specific binding. This resin may be used in routine laboratory work, such as small-scale preparative separations, and particularly micro-preparative separations that involve very small sample volumes.

Several publications[2-4] and dissertations[5-6] have cited use of this product in their research protocols.

Storage/Stability

Store this product, as sold in lyophilized form, at room temperature.

The swollen resin may be stored at 2-8 °C for up to 1 month. For longer-term storage, it is suggested to include an anti-bacterial agent, such as 20% ethanol, in the resuspension buffer.[5]

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Product Summary

Bed volume[7]: 4-6 mL/g dry Sephadex®

DNA exclusion limit[7]: 10 bp

Oligonucleotide exclusion limit[7]: 10 bp

Recommended pH range[7]: 2-13

Swelling time[7]:

- 72 hours at 20 °C
- 5 hours at 90 °C

DNase and RNase: None detected

Details on nuclease testing

The nuclease tests below use supernatant that has been isolated after centrifuging a resuspension of the Sephadex® beads in water at 116 mg beads per 1 mL of water, with overnight incubation at 2-8 °C. Small aliquots of the Sephadex® supernatant are used for the assays. In the course of the nuclease tests, degradation of DNA or RNA was not detected.

Endonuclease-Exonuclease

One µg of λ Hind III fragments was incubated in an aliquot of the Sephadex® supernatant for 16-18 hours at 37 °C in a 50 µL reaction mixture containing 30 mM Trizma®-HCl (pH 7.8), 50 mM NaCl and 10 mM $MgCl_2$. No degradation of the DNA fragments was detected by agarose gel electrophoresis.

Endonuclease (Nickase)

One µg of pBR322 DNA was incubated in an aliquot of the Sephadex® supernatant for 16-18 hours at 37 °C in a 50 µL reaction mixture containing 30 mM Trizma®-HCl (pH 7.8), 50 mM NaCl and 10 mM $MgCl_2$. No conversion of the covalently closed circular DNA to the nicked or linear form was observed by agarose gel electrophoresis.

RNase

Two µg of transfer RNA were incubated in an aliquot of the Sephadex® supernatant for 16-18 hours at 37 °C in a 50 µL reaction mixture containing 30 mM Trizma®-HCl (pH 7.8), 50 mM NaCl and 10 mM $MgCl_2$. No degradation of the tRNA was detected by polyacrylamide gel electrophoresis.

References

1. Janson, J.-C., *Chromatographia*, **23(5)**, 361-369 (1987).
2. Murta, V. *et al*., *J. Neurochem.*, **144(6)**, 748-760 (2018).
3. Knewtson, K.E. *et al*., *ACS Omega*, **4(7)**, 12955-12968 (2019).
4. Horkova, V. *et al*., *Cell Rep*., **30(5)**, 1504-1514.e7 (2020).
5. Ahmad, Aftab, "Interaction of Lysozyme with Antilysozyme Antibody". Aligarh Muslim University (Aligarh, India), Ph.D. dissertation, p. 21 (1995).
6. Kuschak, Theodore L., "c-Myc dependent genomic instability of the *Ribonucleotide Reductase R2* gene". University of Manitoba, Ph.D. dissertation, p. 167 (July 2000).
7. Supplier information.

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