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INSTRUCTION MANUAL

NU-CLEAN D50, Sephadex G50

Spin Columns for the Purification
of Radiolabeled DNA
IB06050

Physical Specifications

Package Quantity	10
Maximum Sample Volume	100 µl
Centrifuge Required	Clinical w/swing bucket rotor

Internal Specifications

Each DNASE- and RNASE-free column is prepackaged with Sephadex G-50 in sterile STE buffer (10mM Tris HCL, 1mM EDTA, pH 8.0). Two autoclavable collection tubes and one non-autoclavable cap are supplied for each column. The optimal sample loading volume is 50µl (100µl Max.) with a maximum of 100µg of nucleic acid per column. Sample should not be viscous prior to loading.
Recovery DNA (>72bp) >90%
Retention Unincorporated NTPs >99%
For best results use a centrifuge with a swinging bucket or horizontal rotor, clinical tabletop centrifuges are also suitable.

Recommended Use

The NU-CLEAN D50, Sephadex G-50 spin columns are intended for use in desalting and removing unincorporated radiolabeled deoxynucleoside triphosphates (dNTPs) from end-label, fill-in, nick-translation, or random primed DNA labeling reactions. This column should be used to purify DNA greater than 72 base pairs in length or RNA greater than 72 base length; smaller DNAs or RNAs will be retained in the column material. After brief centrifugation, the purified nucleic acid is recovered from the column without significant change in volume. For optimum sample recovery and purification when preparing biotinylated probes, use NU-CLEAN spin columns with the “B” designation.
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Quality Assurance

Each lot of NU-CLEAN D50, Sephadex G-50 have been tested for recoveries and retention. IBI Spin Columns have been found to meet or exceed the above specifications. Each lot is also tested for sterility, and the absence of detectable DNASE and RNASE.

Storage and Stability

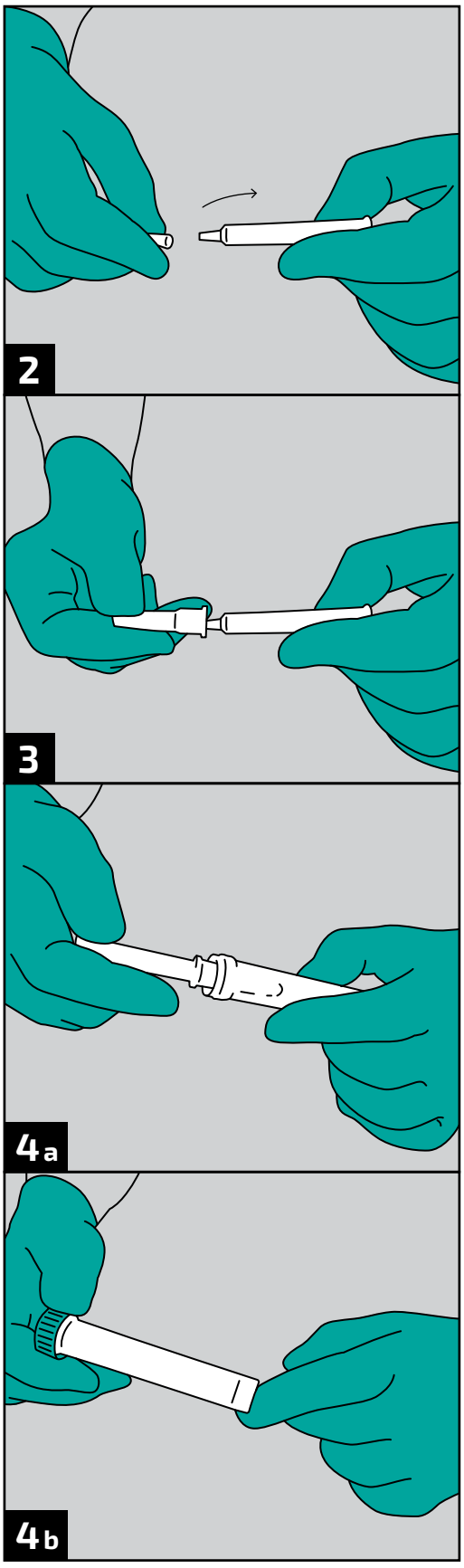
Columns should be stored at 2 – 8°C and are stable for a period of at least one year.
NOTE: DO NOT FREEZE COLUMNS.

References

1. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. "Molecular Cloning: A Laboratory Manual", 2nd Edition. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY p. E.37- E.38.
2. Sephadex®, Pharmacia, Inc., Piscataway, NJ.

Protocol

NOTE: Please read the entire protocol before using spin columns



Steps 2, 3 and 4 are illustrated

1) Invert the column several times to suspend the gel.

2) Remove the small white cap from the output end of the column.

3) Place column inside one of the included collection tubes.

4) Insert assembled column and collection tube into 10ml or 15ml centrifuge tube and fasten cap.

5) Place assembled tube with column into a swinging bucket or horizontal rotor centrifuge and centrifuge for 1 minute at 1100 x g.

6) Remove carrier tube containing spin column from centrifuge and dis-assemble. Remove the collection tube, containing buffer, from the column and discard. (If needed, discard buffer in collection tube and re-assemble column and centrifuge tube and spin again for 1 minute at 1100 x g)

7) Take a new collection tube and place on column.

8) Remove the large cap on input end of column and pipet your sample into the center of the shrunken gel bed inside the column.

9) Re-assemble column, collection tube with centrifuge tube and place in centrifuge.

10) Centrifuge at 1100 x g for 4 minutes.

11) The labeled nucleic acid will be recovered inside the collection tube in approximately 50µl of STE buffer.

12) Greater than 90% of the unincorporated dNTPs will be retained in the column gel, discard the used column in an appropriate fashion.

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