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# INSTRUCTION MANUAL

## MIDI SELECT-D TE, G-25

IB06010

Microcentrifuge Spin Columns for
the Purification of Radiolabeled DNA & RNA

# Physical Specifications

**Package Quantity:** 50
**Maximum Sample Volume:** 50µl
**Centrifuge Type Required:** Micro or Table Top.

# Internal Specifications

Each DNASE- and RNASE-free column is prepackaged with Sephadex G-25 in sterile TE buffer (10mM Tris HCL, 1mM EDTA, pH 8.0). Two nuclease-free collection tubes (autoclavable) are supplied for each column. Proper precautions should be taken to avoid contamination of the column, column contents, collection tubes and samples with exogenous RNASE. The supplied columns and collection tubes are sterile and nuclease-free.
The optimal sample loading volume is 50µl with a maximum of 50µg of nucleic acid per column.
Sample should not be viscous prior to loading.
Pd(N)12 >60%
Recoveries Pd(N)19-24 >80%
Large Oligonucleotides >85%
Retention Unincorporated NTPs >90%
For best results use a microcentrifuge with a fixed angle or horizontal rotor capable of 12,000-16,000 x g. Please note that the microcentrifuge must be suitable for use with 2ml microcentrifuge tubes. In particular, the 2ml tubes positioned in the rotor must not contact any part of the microcentrifuge interior.
Use of a closed-bottom rotor compatible of accepting 2ml tubes is recommended.

# Recommended Use

The MIDI SELECT-D TE, G-25 microcentrifuge spin columns are intended for use in desalting, recovering DNA fragment (>12mer), and removing unincorporated radiolabeled deoxynucleoside triphosphates (dNTPs) from small volume 5' end-labeling reactions and fill-in labeling reactions utilizing a DNA polymerase. In addition, the MIDI SELECT-D TE, G-25 spin columns are RNASE-free and can be used for the rapid purification of oligoribonucleotides and RNA away from unincorporated ribonucleotides (rNTPs) and in other related applications. After brief centrifugation, the purified nucleic acid is recovered from the column without significant change in volume.

# Quality Assurance

Each lot of MIDI SELECT-D TE, G-25 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 & Stability

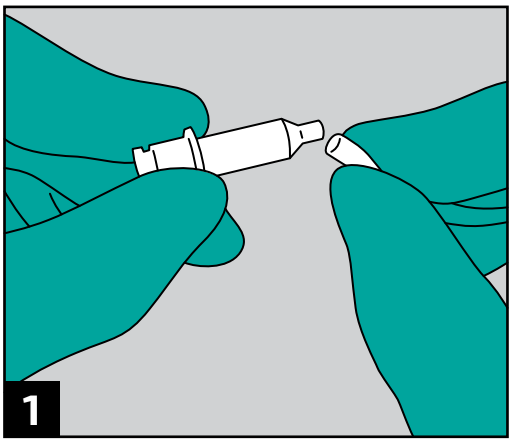
Columns should be stored at 2–8°C and are stable for a period of at least one year.
**NOTE:** DO NOT FREEZE COLUMNS.

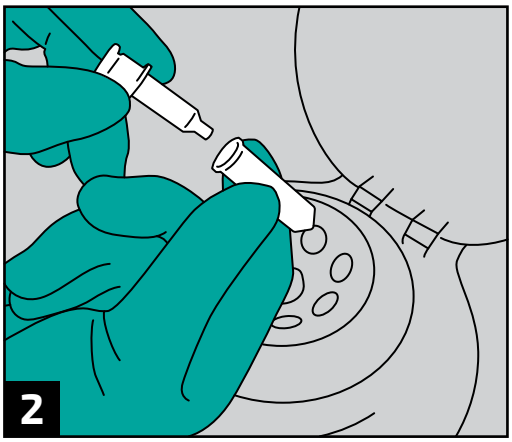
# Reference

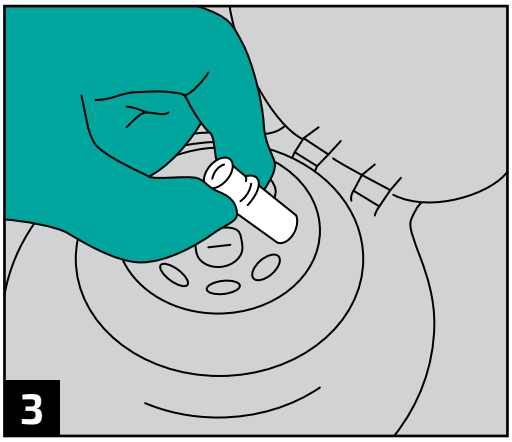
1. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. "Molecular Cloning: A Laboratory Manual",
2nd Edition. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY p. E.37- E.38.
2. Sephadex ®, Pharmacia, Inc., Piscataway, NJ.

# Protocol

**NOTE:** Please read the entire protocol before using spin columns




1) Invert the column several times to re-suspend the gel. Shake the column with a sharp downward motion so as to push all of the gel material form the top of the column to the bottom.

2) To open the output end of the column, break off the tip closure. See Illustration 1.

3) Insert the opened column into one of the collection tubes included in this kit. See Illustration 2.

4) Insert the coupled column and collection tube into a fixed angle or horizontal rotor microcentrifuge, suitable for use with 2ml microcentrifuge tubes. Centrifuge at 12,000–16,000 x g for 30 seconds. See Illustration 3.

5) Once completed, this will have removed any excess buffer in the gel matrix. You may now discard the collection tube containing the buffer.

6) Remove the top cap and lace column into a 2nd collection tube, included in this kit. Carefully pipet a 20ml sample directly into the center of the shrunken gel matrix. Place cap back on top of columns.
Caution: Column caps should be placed back on top of cloumn to prevent any potential contamination during centrifuge procedures.

7) Allow loaded column to sit undisturbed for 2–3 minutes then insert the coupled column and collection tube assembly into the microcentrifuge, so that the slanted gel bed is oriented the same way as it was after the pre-spin.

8) Centrifuge the assembly at 12,000–16,000 x g for 30 seconds.

9) The labeled nucleic acid will be recovered in the collection tubes in approximately 20ml of TE buffer solution.

10) Greater than 90% of the unicorporated NTP(s) may be retained in the column gel. Discard the used column.

**NOTE:** This purification column is intended for ONE purification use only!

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