IB06015 - MIDI SELECT-D TE, G-50

Microcentrifuge Spin Columns for the Purification of Radiolabeled DNA & RNA

For research use only

Package Quantity: 50

Maximum Sample Volume: 50µl

Centrifuge Type Required: Tabletop

INTENDED USE

The MIDI SELECT-D TE, G-50 microcentrifuge spin columns are intended for use in desalting, recovering DNA fragment (>72bp, smaller DNAs will be retained), 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-50 spin columns are RNASE-free and can be used for the rapid purification of RNA (>72bases) 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.

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.

SPECIFICATIONS

Each DNASE- and RNASE-free column is prepackaged with Sephadex G-50 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.

Recoveries	DNA (>72bp)	>90%
	RNA (>72bases)	>80%
Retention	Unincorporated NTPs	>95%

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.

QUALITY ASSURANCE

Each lot of MIDI SELECT-D TE, 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.

PROTOCOL

NOTE: Please read entire protocol before using columns.

1. Invert column several times to fully resuspend the gel. Shake the column with a sharp, downward motion so that no resin remains in the top. Remove top closure (large) first, followed by the bottom closure (small).
2. Place column in one of the collection tubes provided, securely cap column, and centrifuge at 12,000-16,000rpm for 90 seconds in a fixed angle or horizontal rotor microcentrifuge suitable for use with 2ml microcentrifuge tubes.
3. Once completed, discard the collection tube and collected buffer.
4. Place the column in a second collection tube and carefully apply a 50µl sample directly to center of the shrunken gel bed. Secure cap onto column.

   CAUTION: Collection tube caps must be inserted firmly and securely into columns to prevent potential sample loss and microcentrifuge contamination.

5. Allow loaded column to sit undisturbed for 2-3 minutes and then place the spin column/collection tube assembly in the centrifuge so that the slanted bed is orientated the same way as it was after the pre-spin.
6. Centrifuge the assembly at 12,000-16,000 x g for 90 seconds.
7. The labeled nucleic acid will be recovered in the collection tube in approximately 50µl of TE buffer.
8. Greater than 90% of the unincorporated NTP(s) may be retained in the column gel, discard the used column in appropriate fashion.

NOTE: MIDI SELECT-D TE, G-50 columns are intended for one purification usage only.

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.