IB06080 - NU-CLEAN R50, Sephadex G-50

Spin Columns for the Purification of Radiolabeled DNA

For research use only

Package Quantity: 10

Maximum Sample Volume: 100µl

Centrifuge Type Required: Clinical w/Swing Bucket Rotor

INTENDED USE

The NU-CLEAN R50, 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.

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

NOTE: Forceps may be needed to remove the column and collection tubes from the swinging bucket or carrier centrifuge tubes utilized.

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.

QUALITY ASSURANCE

Each lot of NU-CLEAN R50, 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.

PROTOCOL

NOTE: Please read entire protocol before using columns.

1. Invert column several times to fully resuspend the gel. Remove top closure (large) first, followed by the bottom closure (small). We recommend placing columns in a test tube rack or similar device while draining.
2. Place column in one of the collection tubes provided, securely cap column, and centrifuge at 1,100 x g for 1 minute in a swinging bucket or horizontal rotor centrifuge. A larger centrifuge tube may be used as a carrier for the column/collection tube assembly.
3. Empty the collection tubes of buffer and place columns back into the same collection tubes.
4. Centrifuge at 1,100 x g for 1 minute at speed. Discard collection tube and collect buffer.
5. Place column in a second collection tube, carefully apply a 50µl sample directly into the center of the shrunken gel bed, and centrifuge at 1,100 x g for 4 mintues at speed.
6. The labeled nucleic acid will be recovered in the collection tube in approximately 50µl of STE buffer.
7. As greater than 90% of the unincorporated dNTPs will be retained in the column gel, discard the used column in an appropriate fashion.

NOTE: NU-CLEAN R50, Sephadex 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.