

# IB06065 - NU-CLEAN D25, Sephadex G-25

Spin Columns for the Purification of Radiolabeled DNA

*For research use only*

**Package Quantity:** 25  
**Maximum Sample Volume:** 100 $\mu$ l  
**Centrifuge Type Required:** Clinical w/Swing Bucket Rotor



## **INTENDED USE**

The NU-CLEAN D25, Sephadex G-25 spin columns are intended for use in desalting, recovering DNA fragments (>12mer), and removing unincorporated radiolabeled deoxynucleoside triphosphates (dNTPs) from end-labeling reactions and fill-in labeling reactions utilizing a DNA polymerase. 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-25 in sterile STE 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 50 $\mu$ l (100 $\mu$ l Max.) with a maximum of 100 $\mu$ g of nucleic acid per column. Sample should not be viscous prior to loading.

Recoveries	Pd(N)12	>60%
	Pd(N)19-24	>80%
	Large Oligonucleotides	>85%
Retention	Unincorporated NTPs	>90%

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 D25, Sephadex 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.

## **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 at speed 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 tube of buffer and place column back into the original collection tube.
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 50 $\mu$ l sample directly into the center of the shrunken gel bed and centrifuge at 1,100 x g for 4 minutes at speed.
6. The labeled nucleic acid will be recovered in the collection tube in approximately 50 $\mu$ l of STE buffer.
7. 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 D25, Sephadex G-25 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.