

CentriSpin™-20 Columns Data sheet:

Cat #	Product
S5300.0020	CentriSep Accessory Kit
S5300.0100	CentriSep Dye Terminator Removal Kit pack of 100 columns
S5301.1020	CentriSpin™-10 columns (pack of 20) for DNA, RNA and protein purification
S5301.2020	CentriSpin™-20 columns (pack of 20) for DNA, RNA and protein purification
S5301.2050	CentriSpin™-20 columns (pack of 50) for DNA, RNA and protein purification
S5301.4020	CentriSpin™-40 columns (pack of 20) for DNA, RNA and protein purification

Usage / Application:

CentriSpin™-20 columns are used for the fast and efficient purification of larger molecules (proteins, nucleic acids, complex carbohydrates, etc.), from small molecules (nucleotides, buffer salts, etc.). The CentriSpin™-20 gel will provide excellent recovery (> 70%) of DNA fragments **> 20 base pairs or 20-mer**, or proteins, peptides **≥ 25 kDa** while removing > 98 % of salts, NTP's and other unwanted low-molecular-weight impurities.

The column gel is hydrated with reagent grade water or a suitable buffer and spun in a microcentrifuge or swinging-bucket centrifuge to **remove the interstitial fluid**. The sample is then applied and the column is spun again, **processing the sample**. The sample is purified by the retention of low-molecular-weight contaminants in the matrix, while the larger molecules of interest are exchanged into the buffer of choice and eluted into the collection buffer.

These columns are far superior – in **ease of use, speed, and non-toxicity** – to such common techniques as phenol/chloroform extraction, ethanol precipitation, dialysis and ultrafiltration.

Benefits of the CentriSpin™ columns:

- Rapid and efficient separations
- Buffer not pre-selected
- Columns stable at room temperature
- Convenient 20 – 50 µl sample size

Centrifugation Notes:

Maximum yield and efficiency are obtained with the horizontal or swinging-bucket rotors. However, fixed-angle-rotor microcentrifuges provide acceptable performance and save time.

On a variable speed microcentrifuge, DO NOT use the pulse button, which overrides the speed setting and takes the rotor to maximum g-force. If you are not sure of the g-force generated by your centrifuge at specific speeds, calculate the correct speed by using the following formula:

$$\text{rpm} = \sqrt{\text{RCF} / (1.119 \times 10^{-5})} \text{ r(cm)}$$

Where: rpm = revolutions per minute
RCF = Relative Centrifugal Force
r = radius (cm) measured from centre of spindle to bottom of rotor bucket.

Example: RCF = 750 and r = 7.5 cm

$$\text{rpm} = \sqrt{750 / (1.119 \times 10^{-5})} (7.5) = 2990$$

Quality Control:

Every batch of CentriSpin-20 columns is tested for separation efficiency and fill accuracy.

Material provided:

- CentriSpin-20 columns containing dry gel
- Wash tubes (2 ml)
- Sample Collection tubes (1.5 ml)

Additional Materials Recommended

- Microcentrifuge (variable speed)
- Variable pipets
- Vortex mixer

Common Problems

1. Failure to remove excess interstitial fluid after hydration of the columns.
 2. Touching the side of the column during sample application.
- Both errors can result in ineffective separation.

Solutions

1. Note if any columns have released less fluid than the others during the first spin. Simply spinning them again briefly will usually remove the excess fluid.
2. Load the sample directly into the center of the gel bed and do not touch the sample to the walls of the columns

Reference:

Sambrook, J., Fritsch, E.F., and Maniatis, T., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1989.