



CentriSpin and CentriSep Columns “Technical Notes and Trouble shooting”:

Cat #	Product
S5300.0020	Centri Sep Accessory Kit
S5300.0032	Centri Sep Dye Terminator Removal Kit
S5301.1020	Centri Spin 10 columns for DNA, RNA and protein purification
S5301.2020	Centri Spin 20 columns for DNA, RNA and protein purification
S5301.4020	Centri Spin 40 columns for DNA, RNA and protein purification
S5301.0000	Centri Spin Combi Pack for DNA, RNA and protein purification

The Centri Spin columns can be used for the following applications:

- Antibody labelling
- Binding Applications
- Desalting
- Differential Display
- Electroporation
- FISH
- Gene products
- Nick translation
- Probe labelling
- Protein labelling
- Receptor analysis
- Signal transduction
- Solid phase assay
- Transfection
- Transformation studies

PRINCIPLE

CentriSpin-10:

CentriSpin-10 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-10 gel will provide excellent recovery of **DNA fragments > 10 base pairs or 10-mer**, while removing > 98 % of salts, NTP's and other unwanted low-molecular-weight impurities. Proteins, peptides, and protein conjugates **≥ 5 kDa** can also be efficiently separated/desalted from unwanted low-molecular-weight impurities.

CentriSpin-20:

CentriSpin-20 columns provide excellent recovery of oligonucleotide fragments **> 20 base pairs** while removing > 98% of salts, dNTPs, and other unwanted low-molecular weight impurities. Proteins, peptides and their conjugates **> 25 kDa** can also be efficiently desalted or separated from unwanted low molecular weight impurities. CentriSpin-20 are optimal suited for: 1. removal of free and labeled dNTPs from DNA/RNA, as in nick translation, end-labeling and PCR reactions. 2. Primer removal. 3. Removal of hexamers and octamers from primer-walking and random primer labeling. 4. Desalting, buffer exchange, and removal of trace amounts of phenol, as in multiple restriction digests. 5. Desalting / purification / buffer exchange of peptides or proteins **> 25 kDa**.

CentriSpin-40:

CentriSpin-40 columns will retain up to 80% of short oligonucleotides (<25 mer) while providing excellent recovery of up to 70% of large DNA/RNA fragments **> 135 base pairs**. Proteins, peptides and their conjugates **> 100 kDa** can also be efficiently desalted or separated from unwanted low-molecular-weight impurities. Centri Spin 40 columns are optimal suited for: 1. Removal of primers or primer dimers from PCR extension products (>150 kDa). 2. Desalting and purification of proteins **> 100 kDa**. 3. Removal of the following impurities from solution: a) transferrin, b) BSA, c) hemoglobin, d) albumin.

The column design is based on the description of gel filtration for the purification of DNA from nick translation reactions by Sambrook, et.al. Each CentriSpin-10 unit consists of a specially fritted microfuge tube, dry gel, a wash tube and a sample collection tube, all specifically designed for this purpose.

The column gel is hydrated with either reagent-grade water or a suitable buffer, and spun in a micro centrifuge or swinging-bucket centrifuge to remove the interstitial fluid. After your sample is applied, the column is spun again to remove the low-molecular-weight components, leaving you not only with a purified sample, but one exchanged into the buffer of your choice.



GENERAL TECHNICAL PROTOCOL CentriSpin™ Columns

1. Column hydration

add 0.65 ml reagent grade water or appropriate buffer
Replace column cap and vortex vigorously for about 5 seconds
Remove air bubbles, e.g. by sharply tapping at the bottom of the spin column

2. Removal of interstitial fluid

Spin both column and wash tube
Discard tube and interstitial fluid

3. Sample processing

Load sample on top of the gel (as centred as possible)
Place column into collection tube
Spin and collect purified sample

1. Säulenhydratisierung

0,65 ml reines Wasser oder Puffer auf das Säulenmaterial geben
Verschlusskappe wieder aufsetzen und für ca. 5 Sekunden stark vortexen.
Luftblasen, z.B. durch starkes "Klopfen" am Säulenboden entfernen.

2. Entfernen der überschüssigen Flüssigkeit

Beides, Säule und "Waschtube", zentrifugieren.
"Waschtube" und überschüssige Flüssigkeit verwerfen.

3. Probenaufbereitung

Probe auf das Gelbett (möglichst im Zentrum) auftragen
Probensäule in das "Sammeltube" stecken.
Zentrifugieren und gereinigte Probe im "Sammeltube" auffangen.

TECHNICAL NOTES "RNA Purification"

Materials and media supplied are tested for nickase activity, and precautions were taken to prevent the introduction of RNase into the columns. However, we do not certify these columns to be RNase-free, as the column caps cannot be autoclaved. Also, as we provide all columns in a dry-gel format (which contributes significantly to the consistency of column performance) we do not treat them with DEPC.

We can assure that several researchers do use these columns for RNA work. The contact time with the media is extremely short, and we have never had complaints of RNase activity. These researchers apparently follow the normal CENTRI SEP or CENTRI SPIN protocol.

To be absolutely scrupulous, one may do the following:

1. Observe normal RNA precautions

- a: use sterile plastic ware
- b: use baked glassware
- c: Use RNase-free glass ware and treat the column caps accordingly

2. Hydrating procedure

- a: hydrate columns using DEPC treated water or buffer (100 µl DEPC per litre)
- b: while the column is hydrating, remove the cap and autoclave the column

3. Centrifugation

- a: Spin the column to remove excess interstitial fluid and continue with the normal CentriSep or CentriSpin protocol (step 3 of the general technical protocol).

However, as we did not design these columns for this purpose, we still do not guarantee fitness-for-use.



TECHNICAL NOTES “Protein Purification”

Maximizing sample recovery when using CentriSpin or CentriSep columns for desalting purposes.

Protein Interaction: Reduced recovery of protein samples may occur when using CentriSpin or CentriSep columns. This is due to a specific or non-specific interaction with the column matrix. Below is a list of common modes of interaction and suggestions for minimizing their effect on sample recovery.

Using the guide below, modify your CentriSpin or CentriSep hydration buffer to reduce your suspected interaction. In addition, check that the molecular weight of your sample exceeds the exclusion limit of the CentriSpin or CentriSep column used.

- 1. Hydrophobic Interaction:** Hydrophobic proteins in high molarity chaotropic salts solutions may adsorb to the CentriSpin and CentriSep matrix. Subsequent elution of the protein with water is indicative of a hydrophobic interaction.

Possible Solution(s)

- Decrease ionic strength of hydration buffer.
- Increase pH of your hydration buffer.
- Add organic solvent to your hydration buffer (i.e. 5 % isopropanol or ethanol).
- Add 10 % ethylene glycol to your hydration buffer.
- Add a suitable non-ionic detergent to about 0.1 % (i.e. Tween, Triton, Nonidet P40, Brij, Span, Lubrol).

- 2. Ionic Interactions:** The CentriSpin matrix has a slight net negative charge at neutral pH. Positively charged molecules may be slightly retained.

Possible Solution

- Increase the ionic strength of your hydration buffer (> 50 mM)

- 3. The Sample Precipitates on Column:** Precipitation may be caused by salt removal, sample dilution, or a change in pH.

Possible Solution

- Increase the ionic strength of your hydration buffer
Protease Degradation Add a suitable inhibitor

- 4. Lectin binding:** Carbohydrate binding proteins may adsorb to the column matrix.



TECHNICAL NOTES “Volumes greater than those recommended”

CentriSpin columns will process sample sizes up to 50 µl, and CentriSep up to 100 µl. If the volume to be processed exceeds these sizes, please use a concentration protocol to reduce the volume of your sample prior to using the column.

Concentration of protein samples:

Pellet your protein using ammonium sulfate (50%), then rehydrate in a weak ionic solution (20 mM phosphate, or just water.) Then use CentriSpin or CentriSep to quickly and completely remove the ammonium sulfate and all other salts and small contaminants.

Concentration of DNA samples: DNA samples greater than 50 µl prior to purification using CentriSpin Columns or concentration of DNA samples greater than 100 µl prior to purification using CentriSep Columns. Adapted from Maniatis, Fritsch, and Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1982, page 465.

Secondary Butanol Protocol

During extraction of aqueous solutions with solvents such as secondary butyl alcohol (2-butanol) or n-butyl alcohol (1-butanol), some of the water molecules, but not DNA or solutes, become partitioned into the organic phase. By carrying out several cycles of extraction, the volume of a DNA solution can be significantly reduced. This method of concentration DNA is used to reduce the volume of dilute DNA solutions to the point where the DNA can be easily recovered by either precipitation with ethanol, or by use of CentriSpin and CentriSep Columns.

1. Add an equal volume of 2-butanol to the DNA sample and mix well.

Note: addition of too much 2-butanol can result in removal of ALL the water and premature precipitation of the DNA.

2. Centrifuge at 1600 g for 1 minute. Remove and discard the upper (2-butanol) phase.
3. Repeat steps 1 and 2 until the desired volume is achieved.
4. Extract the sample twice with water-saturated ether to remove the 2-butanol. Remove the ether by evaporation.
5. Because 2-butanol extraction does not remove salt, the salt concentration increases in proportion to the reduction in volume of the solution. Therefore, the buffer concentration must be adjusted by either dialysis, ethanol precipitation, or by the quick and efficient use of a CentriSpin or CentriSep Column.

TECHNICAL NOTES “Taq-Polymerase Removal”

Regarding the removal of polymerase following PCR, some options are listed below. (Our R&D group did not consider heat inactivation to be practical.)

1. Do nothing. It's usually not necessary to remove polymerase for most downstream steps.
2. Denature the polymerase with SDS. Remove the SDS with a spin column (CentriSpin-20 or CentriSep). Some re-naturation may occur.
3. Digest the polymerase with Proteinase K (ca. 27 kD) and denature the Proteinase K with SDS @ 0.1%. Remove the Proteinase K and SDS with a spin column (CentriSpin-40).
4. Inactivate the polymerase by adding phosphate or EDTA (this is a reversible reaction.)
5. Extract the polymerase using phenol/chloroform. Remove the phenol/chloroform with a spin column (CentriSpin-20 or CentriSep).