Takara Bio USA

CHROMA SPIN™ Columns User Manual

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I. Introduction

A. Summary

Spin-column chromatography, which combines economy, effectiveness of gel filtration, and speed of centrifugation, is a common method of choice for nucleic acid purification. **CHROMA SPIN Columns** are routinely used to purify DNA or RNA from smaller contaminants such as salts, solvents, nucleotides, enzymes, proteins, polysaccharides, metabolites, and in some cases other (smaller) nucleic acids. In addition, CHROMA SPIN columns can be used for size selection of nucleic acids within a wide size range (from 15 nucleotides to >1,000 bp). Nucleic acids purified with these columns are well suited for use in many molecular biology experimental procedures, including sequencing, labeling, PCR, cloning, and *in vitro* transcription (Sambrook and Russell 2001).

CHROMA SPIN columns are based on the principle of gel filtration chromatography and are packed with resins designed to selectively remove contaminating molecules based on size. The proprietary resins are comprised of uniform microscopic beads of a hydrophilic porous material. Molecules larger than the pore size are excluded from the resin. These molecules quickly move through the gel bed when the column is centrifuged briefly, while molecules smaller than the pore size are held back (Hagel et al. 1989; Porath et al. 1959). Thus, DNA and protein molecules are eluted from the column in order of decreasing molecular size. Highest sample recovery (>90%) is achieved when the molecule of interest is significantly larger than the matrix pore size; highest purity (>90% enrichment) is achieved when contaminants are significantly smaller than the matrix pore size.

These columns are available in six matrix pore sizes that cover a wide range of applications. The number at the end of each column name indicates the approximate 50% retention point. The smallest pore-size column, CHROMA SPIN-10, allows the selective removal of molecules as small as NaCl or dNTPs; CHROMA SPIN-1000, the largest pore-size column, will remove 50% of molecules as large as 1,000-bp DNA fragments. In addition, there are four intermediate pore-size columns, CHROMA SPIN-30, -100, -200, and -400, which can be used for the removal of intermediate-size nucleic acids and proteins. There is no defined upper size limit of nucleic acid that can be purified. We have successfully used these columns to purify yeast genomic DNA (~79 kb). Please refer to Appendix B for additional information on purification and removal characteristics of the various pore-size columns.

CHROMA SPIN columns are provided sterilized and ready to use, with a choice of three different column buffers (TE, STE, and DEPC-treated H_2O). In many cases, pre-equilibrating the column before use is not required (Table I); however, the column matrix may be re-equilibrated with any buffer that is appropriate for your application. Nucleic acid purification can be accomplished in just a few steps (Figure 1), and the entire procedure takes only ~9 min, including pre-spin time. The purified molecules are eluted in sterile column buffer and automatically collected in a self-contained, nuclease-free storage tube.

Table I. Buffers and Applications

Buffer	Application
TE (10 mM Tris-HCl [pH 8.0], 1.0 mM EDTA)	Routine DNA work
STE (TE buffer + 0.1 M NaCl)	Purification of plasmid DNA and other applications requiring high salt concentration
DEPC-water (water and matrix treated with 0.1% DEPC, autoclaved)	A nuclease-free buffer for RNA work

There is no need to transfer the eluted nucleic acid sample after purification—this reduces the risk of spills, losses, and accidental exposure in the case of radioactive samples. (Unincorporated radioactive nucleotides are safely retained in the small volume of the column.)

The separation performance of each CHROMA SPIN column has been vigorously tested with a wide range of nucleic acid fragments (Figure 3, Appendix B). All components are sterilized including the column, buffer, gel-filtration matrix, and collection tubes. Also, each new lot is tested to verify that it is nuclease-free.

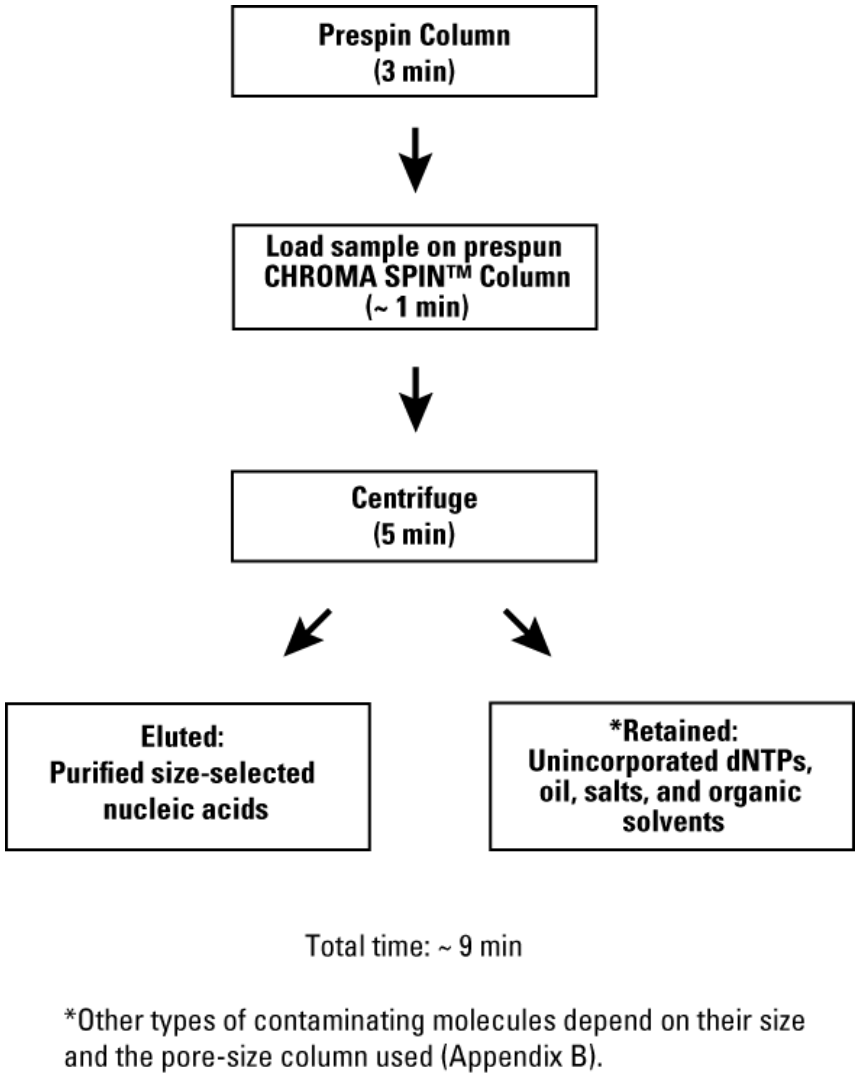

Figure 1. Nucleic acid purification using CHROMA SPIN Columns.

B. Applications

The following section provides several applications using CHROMA SPIN columns.

Use any CHROMA SPIN Column for the following applications:

- Desalting or exchanging nucleic acid/protein solution buffer
- Removing oils and solvents (such as phenol)
- Removing unincorporated nucleotides, whether they are unlabeled or labeled (Radioactively labeled, biotinylated, and fluorescently-labeled nucleotides are all efficiently removed.)
- Removing all other molecules smaller than 5 kDa

Use CHROMA SPIN-10 Columns for the following applications:

- Purifying small oligonucleotides (>15 bp) after labeling reactions

Use CHROMA SPIN-30 Columns for the following applications:

- Purifying DNA and RNA molecules (>35 bp) after labeling reactions
- Purifying proteins >30 kDa
- Removing short primers (<9 bp) after labeling or cDNA synthesis
- Removing unligated DNA linkers (<9 bp) after ligation reactions

Use CHROMA SPIN-100 Columns for the following applications:

- Recovering DNA molecules >140 bp at a high yield
- Purifying DNA molecules >100 bp after PCR amplification (high purity)
- Purifying RNA molecules >140 bp after *in vitro* transcription
- Eliminating unextended primers, short extension products, and digested DNA fragments <30 bp
- Removing enzymes and proteins <250 kDa
- Removing digested agarose polymers

Use CHROMA SPIN-200 Columns for the following applications:

- Recovering DNA molecules >350 bp at a high yield
- Purifying DNA molecules >150 bp after PCR amplification (high purity)
- Removing PCR primers <50 bp and primer-dimers <45 bp
- Removing proteins, including enzymes <1,000 kDa

Use CHROMA SPIN-400 Columns for the following applications:

- Purifying (or size-selecting) DNA molecules >600 bp after PCR amplification or cDNA synthesis
- Purifying small plasmids >600 bp
- Removing larger primers, extension products, and digested DNA fragments <100 bp
- Size-selecting larger mRNAs by removing short (<100 nucleotides) RNA molecules prior to cDNA synthesis and cloning
- Removing proteins, including enzymes <8,000 kDa

Use CHROMA SPIN-1000 Columns for the following applications:

- Purifying DNA molecules >1,350 bp (including λ DNA, plasmids, and yeast genomic DNA)
- Size-selecting large cDNAs (>1,350 bp) for library construction
- Removing RNA and DNA molecules <300 bp
- Removing all proteins, including enzymes

NOTE: For additional information on choosing the correct matrix pore size, see Appendix B.

II. List of Components

Store all CHROMA SPIN products at room temperature (10–32°C). Do not freeze. Columns are packed in sterile, sealed plastic bags.

CHROMA SPIN+ STE Columns
Cat. Nos. 636055 & 636060

- 20 CHROMA SPIN+STE Columns
  Column matrix has been equilibrated and stored in sterile STE buffer
  (0.1 M NaCl, 10 mM Tris-HCl [pH 8.0], 1 mM EDTA)
- 40 Sterile 2-ml Microcentrifuge Collection Tubes

CHROMA SPIN+ STE Columns
Cat. Nos. 636056, 636058 & 636061

- 50 CHROMA SPIN+STE Columns
  Column matrix has been equilibrated and stored in sterile STE buffer
- 100 Sterile 2-ml Microcentrifuge Collection Tubes

CHROMA SPIN+ TE Columns
Cat. No. 636072

- 20 CHROMA SPIN+TE Columns
  Column matrix has been equilibrated and stored in sterile TE buffer
  (10 mM Tris-HCl [pH 8.0], 1 mM EDTA)
- 40 Sterile 2-ml Microcentrifuge Collection Tubes

CHROMA SPIN+ TE Columns
Cat. Nos. 636066, 636069, 636073, 636076, 636079 & 636082

- 50 CHROMA SPIN+TE Columns
  Column matrix has been equilibrated and stored in sterile TE buffer
- 100 Sterile 2-ml Microcentrifuge Collection Tubes

CHROMA SPIN + DEPC-H_2O Columns
Cat. Nos. 636089, 636093 & 636096

- 20 CHROMA SPIN + DEPC-H_2O Columns
  Column matrix has been equilibrated and stored in sterile H_2O +
  0.1 mM EDTA (pH 8.0) treated with diethyl-pyrocarbonate (DEPC), and ther
  autoclaved.
- 40 Sterile 2-ml Microcentrifuge Collection Tubes

CHROMA SPIN + DEPC-H_2O Columns
Cat. Nos. 636087 & 636090

- 50 CHROMA SPIN + DEPC-H_2O Columns
  Column matrix has been equilibrated and stored in sterile H_2O +
  0.1 mM EDTA (pH 8.0) treated with diethyl-pyrocarbonate (DEPC), and ther
  autoclaved.
- 100 Sterile 2-ml Microcentrifuge Collection Tubes

III. CHROMA SPIN Column Protocol

A. General Considerations

- If you wish to replace the column storage buffer with a different buffer, see Section III.D.
- We recommend centrifuging CHROMA SPIN Columns in a swinging bucket or horizontal rotor to allow the sample to pass through the column matrix uniformly. Fixed-angle rotors can also be used, but there is a risk that a portion of the sample will slide down the inner side of the column instead of passing through the gel matrix, resulting in reduced or inconsistent purification.
- To determine the rpm appropriate for your centrifuge rotor, see Appendix A. Alternatively, a clinical (tabletop) centrifuge can be used.

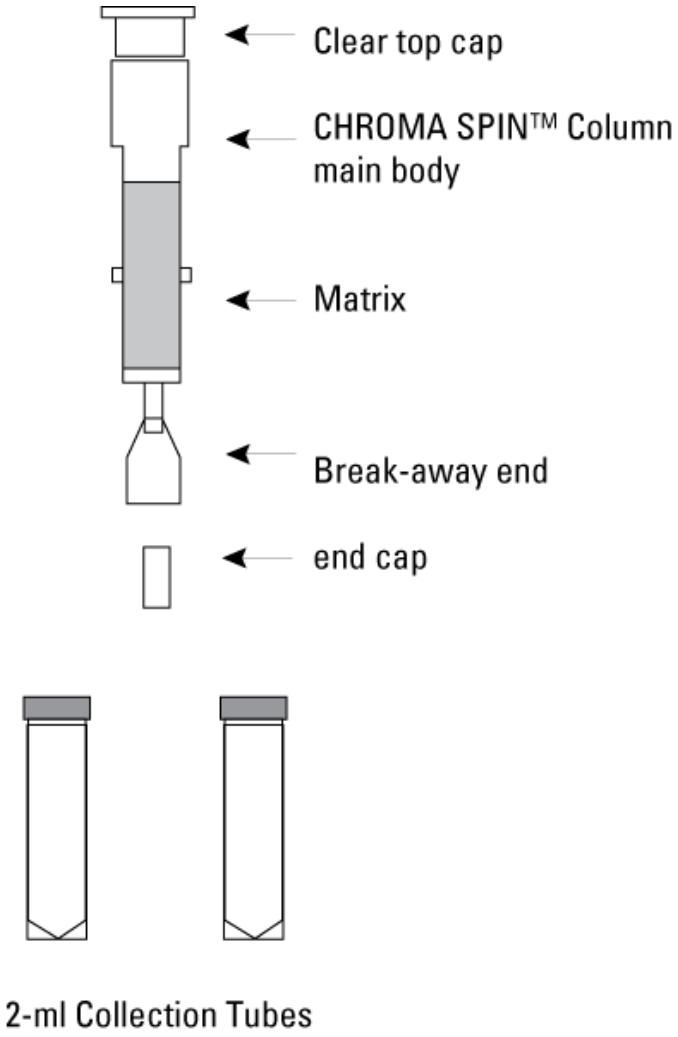

Figure 2. Standard CHROMA SPIN column and collection tubes.

B. Column Capacity

1. **Sample concentration**

   Typical molecular biology applications involve concentrations of nucleic acids that are well below the saturation limit for the resin in CHROMA SPIN Columns. Nevertheless, there is a recommended upper limit on the concentration of protein or nucleic acid in the sample to be purified (Table II). The upper concentration limits are determined by three factors: (1) the size of the molecules, (2) the potential for random aggregation, and (3) the viscosity of the sample. If the concentration exceeds the recommended limits indicated in Table II, there is a risk that the molecules (a) will not be completely dissolved, (b) will form aggregates, or (c) will form a highly viscous solution. Any one of these factors or a combination thereof will reduce the effectiveness of the column by impeding the progress of large molecules through the gel bed and/or preventing small particles from entering the matrix pores.

   NOTES:

   - If your sample is above the recommended maximum concentration and you notice a problem with viscosity, aggregation, or incomplete dissolution, then dilute the sample.
   - If the DNA or RNA in the sample to be purified is below the recommended minimum concentration, add carrier (e.g., sonicated calf thymus DNA, glycogen, or tRNA) to a final concentration of 0.02–0.1 mg/ml. This will improve recovery of the desired molecules from the column.

2. **Sample volume**

   The volume of sample that a CHROMA SPIN Column can accommodate depends on the type (pore size) of column matrix (Table III). It is important to not exceed the maximum recommended sample volume for each column; excessive sample volume may cause small molecules, normally retained in the matrix pores, to be eluted. Each column also requires that a minimum sample volume be loaded for efficient recovery of purified molecules. If the sample volume is below the recommended minimum, there is a risk that some of the desired large molecules will not be efficiently recovered.

   NOTES:

   - If the sample volume is less than the recommended minimum volume, dilute the sample before loading it on the column—or load the undiluted sample onto the prespun column and apply an additional aliquot of buffer to make up the difference between the sample volume and the recommended minimum volume.
   - Please see Appendix B for additional information on how matrix pore size influences column performance.

Table II. Recommended Sample Concentrations for Various Types of Molecules

Molecules in the sample	Optimum concentration range
NTPs, dNTPs	up to 4 mM each
DNA (up to 50 kb)	0.02–1.0 mg/ml
DNA or RNA (<100 bp)	0.02–1.5 mg/ml
Protein (>30,000 Da)	<500 µM (<20 mg/ml)

Table III. Range of Sample Volumes for Chroma Spin Columns

Column (standard capacity)	Sample volume
CHROMA SPIN-10, -30	25–50 µl
CHROMA SPIN-100, -200	40–75 µl
CHROMA SPIN-400, -1000	70–100 µl

C. Purification using CHROMA SPIN Columns

1. Upon removing a CHROMA SPIN Column from the protective plastic bag, invert it several times to resuspend the gel matrix completely.
2. Holding the CHROMA SPIN Column upright, grasp the break-away end between your thumb and index finger and snap off (Figure 2). Place the end of the spin column into one of the 2-ml microcentrifuge (collection) tubes provided, and lift off the top cap. Save the top cap and the end cap.
3. Centrifuge at 700*g* for 5 min. After centrifugation, the column matrix will appear semi-dry. This step purges the equilibration buffer from the column and reestablishes the matrix bed.
4. Remove the spin column and collection tube from the centrifuge rotor, and discard the collection tube and column equilibration buffer.

   NOTE: Always hold both the CHROMA SPIN Column and the 2-ml microcentrifuge tube when removing them from the rotor.
5. Place the spin column into the second 2-ml microcentrifuge tube. Carefully and slowly apply the sample to the center of the gel bed's flat surface. Do not allow any sample to flow along the inner wall of the column. (Refer to Table III for recommended sample volumes.)

   NOTE: For particularly small sample volumes (e.g., <30 µl), a conventional, tapered 1.5-ml microcentrifuge tube can be substituted for the 2-ml collection tube. This will allow the sample to be confined to a narrower area for easier handling.
6. Centrifuge at 700*g* for 5 min.
7. Remove the spin column and collection tube from the rotor and detach them from each other. The purified sample is at the bottom of the collection tube.

   NOTE: Hold the sample collection tube to prevent it from detaching from the spin column. This is particularly important when radioactive samples are processed. For radioactive samples, seal the used spin column with the top and end caps prior to transfer and disposal.

D. Buffer Exchange

For those applications in which none of our column storage buffers are convenient for storing the purified nucleic acid sample, the column matrix may be re-equilibrated with a more appropriate buffer for the particular application.

1. Invert the CHROMA SPIN Column several times to completely resuspend the gel matrix.
2. Remove the top cap first and then the bottom cap from the column. Save the caps. Place the bottom tip of the column gently (snugly, but not tightly) into one of the 2-ml microcentrifuge tubes provided.
3. Discard any buffer that immediately collects in the 2-ml microcentrifuge tube. Replace the column gently into the same microcentrifuge tube.
4. Centrifuge the column in a swinging bucket rotor or in a fixed-angle rotor at 700*g* for 3 min.
5. Discard the collected buffer from the 2-ml tube. Replace the column gently in the tube. Add 1 ml of the new buffer to the gel. After the first application of the new buffer, replace both caps on the column and resuspend the matrix completely by inverting the column several times.
6. Let the buffer drain through the column by gravity or centrifuge again at 700*g* for 3 min.
7. Empty the 2-ml collection tube and replace the column gently in the same 2-ml tube. Repeat the buffer application and centrifugation twice more.

   NOTE: After the first application and removal of buffer, do not invert the column or resuspend the matrix because disrupting the matrix bed will reduce the final yield.

8. If the column is going to be used right away, remove the buffer according to Section III.C, Steps 3 & 4, and proceed with purification (Section III.C.5–7).

   NOTE: If the column will be saved for later use, close the column with the bottom cap and add 0.7 ml of the new buffer. Replace the top cap tightly, and invert the column several times to resuspend the gel matrix. Store the column at 4°C or room temperature (22°C) in an upright position. **Do not freeze.**

E. Additional Hints

- The column should be in an upright position at all times (except when centrifuging) to avoid sample back-flow.
- The column fitted in the 2-ml microcentrifuge tube can be placed inside a 17 x 100-mm polypropylene tube during centrifugation in a swinging bucket rotor for ease of handling.
- For easier sample loading on the gel matrix, add Bromophenol Blue (0.01% w/v) to the sample. The dye molecules will be removed by the column, along with other small molecules.
- Do not overload or underload the column. For optimal column performance, follow the recommended sample volumes, as described in Section III.B.
- To remove small primers from larger DNA products or templates that contain sequences complementary to the primers, heat-denature the sample before loading it on the column. This will reduce hybridization between primers and larger DNAs and result in better separation.
- For best separation results, keep the sample concentration within the recommended range described in Section III.B. If necessary, dilute the sample or add carrier.

IV. References

Hagel, L., Lundstrom, H., Andersson, T. and Lindblom, H. Properties, in theory and practice, of novel gel filtration media for standard liquid chromatography. *J. Chromatography* **476**:329–344 (1989).

Porath, J. and Flodin, P. Gel filtration: a method for desalting and group separation. *Nature* **183**:1657–1659 (1959).

Sambrook, J. & Russell, D. W. (David W. *Molecular cloning : a laboratory manual*. (Cold Spring Harbor Laboratory Press, 2001).

Appendix A: Conversion of RCF to rpm

Revolutions per minute (rpm) calculation for different rotor sizes:

$$\text{rpm} = 1{,}000 \times \sqrt{}$$

or

$$\text{RCF} = \left(\frac{\text{rpm}}{1{,}000}\right)^2 \times 1.12\ r$$

RCF = relative centrifugal field (g Force = RCF x g)

r = radius (in mm)

Examples:

a. Swinging bucket rotor: Beckman Model TJ-6, r = 125 mm
rpm = **2236** for RCF = 700 (i.e., 700 x g)

b. Fixed-angle rotor: Eppendorf Model 5415 C, r = 50 mm
rpm = **3536** for RCF = 700 (i.e., 700 x g)

Appendix B: Column Performance

For each type of column, the size of the matrix bed, the sample volume, and the conditions of centrifugation (speed and time) have been optimized for maximum purification and minimum loss of the desired sizes of nucleic acids. Table IV lists the cutoff sizes of protein molecules that are removed by CHROMA SPIN-10, -30, -100, -200, and -400 Columns at 90% efficiency (no data is available on the upper limit of the size of proteins purified using CHROMA SPIN-1000). Table V lists cutoff sizes of nucleic acids that are removed by the various columns at efficiencies of 90% and 99%. Table VI lists the cutoff sizes of nucleic acid molecules that are recovered in the eluate at efficiencies of 50, 70, and 90%. Figure 3 shows the overall retention and recovery characteristics of the various columns.

Table IV. Size of Protein Molecules Retained by Various Columns

Column	Estimated MW of proteins removed (daltons); greater than 90% removal
CHROMA SPIN-10	<5,000
CHROMA SPIN-30	<30,000
CHROMA SPIN-100	<250,000
CHROMA SPIN-200	$<1 \times 10^6$
CHROMA SPIN-400	$<8 \times 10^6$

Table V. Size of Nucleic Acid Molecules Removed by the Column Matrix

	Removal: percent of input molecules removed from the sample	
Column	>90%	>99%
CHROMA SPIN-10	<4 bp (NTPs, dNTPs)	NaCl
CHROMA SPIN-30	<17 bp	<9 bp
CHROMA SPIN-100	<50 bp	<30 bp
CHROMA SPIN-200	<70 bp	<50 bp
CHROMA SPIN-400	<170 bp	<100 bp
CHROMA SPIN-1000	<420 bp	<300 bp

Table VI. Size of Nucleic Acid Molecules Recovered in the Eluate

	Recovery percent of input molecules recovered in the eluate		
Column	>50%	>70%	>90%
CHROMA SPIN-10	10 bp	>15 bp	>30 bp
CHROMA SPIN-30	30 bp	>35 bp	>75 bp
CHROMA SPIN-100	100 bp	>140 bp	>230 bp
CHROMA SPIN-200	200 bp	>300 bp	>450 bp
CHROMA SPIN-400	400 bp	>600 bp	>950 bp
CHROMA SPIN-1000	1,000 bp	> 1,350 bp	> 2,000 bp

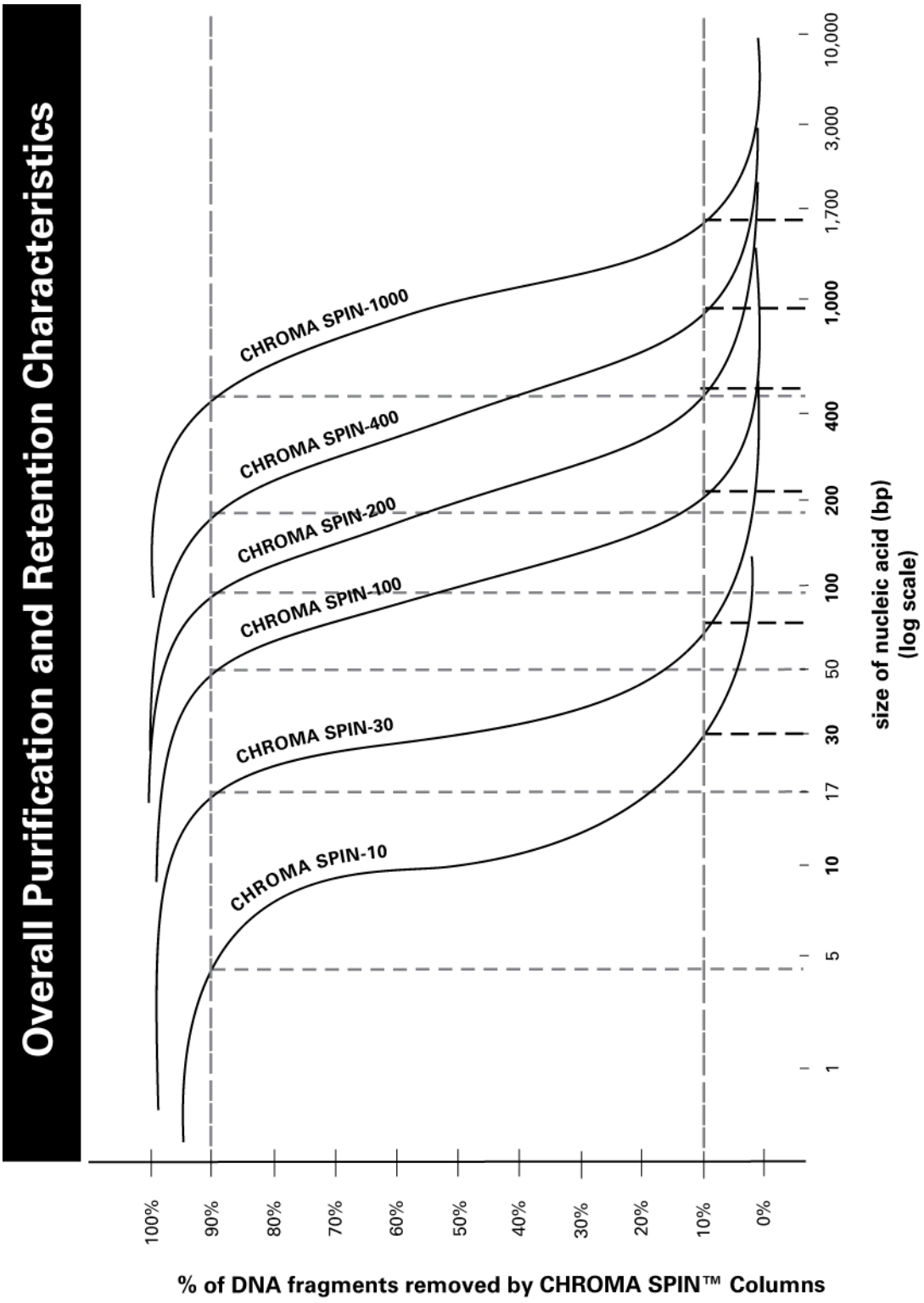

Figure 3. The percentage removal of dsDNA fragments as a function of size for each of the five pore-size CHROMA SPIN Columns. Sample volumes used to generate the curves were in the middle of the recommended ranges.

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