# nucleic acid purification

## Bio-Spin<sup>®</sup> 6 and 30 Columns

#### Introduction

Bio-Spin chromatography columns are ready to use for rapid and efficient cleanup and purification of nucleic acids and proteins using a swinging bucket centrifuge.

#### **Bio-Spin 6 and 30 Columns**

- Remove dye terminators
- Remove unincorporated nucleotides
- Desalting and buffer exchange

The columns are packed with special grades of Bio-Gel<sup>®</sup> P polyacrylamide P-6 or P-30 gel matrices manufactured specifically for Bio-Rad spin columns. This unique gel produces very efficient, noninteractive size separations. We recommend Bio-Spin 6 Tris columns for buffer exchange and desalting applications, while Bio-Spin 30 Tris columns are optimal for removal of unincorporated nucleotides. Bio-Spin columns are suitable for use with 2.0 ml microcentrifuge tubes or 12 x 75 test tubes and are completely autoclavable.

### **Technical Information**

#### Gel Matrix

Bio-Gel P-6 or P-30 polyacrylamide gel suspended in 1.0 ml of buffer.

#### Buffers

SSC buffer (150 mM sodium chloride, 17.5 mM sodium citrate, pH 7.0) with 0.02\% sodium azide.

Tris buffer (10 mM Tris-HCl, pH 7.4) with 0.02% sodium azide.

#### Sample Application Volumes

Nucleic acids, proteins, and peptides, 20-100 µl.

#### **Exclusion Limits**

Bio-Gel P-6 gel: 5 base pairs (nucleic acids) or molecular weight 6,000 (proteins, peptides).

Bio-Gel P-30 gel: 20 base pairs (nucleic acids) or molecular weight 40,000 (proteins, peptides).

#### **Expected Retention and Recovery**

Up to 99% retention of unincorporated nucleotides. Up to 95% recovery of applied DNA.

#### Centrifuge Type

Swinging bucket centrifuge with a centrifugal force of 1,000 x g.

#### Autoclavability

Bio-Spin columns, Bio-Gel P gel, and collection tubes are completely autoclavable at 121°C for 30 min at pH 6.0–8.0.

#### **Chemical Stability**

pH 2–10, common aqueous buffers, formamide, dilute organic acids, alcohol, 20% (v/v) other chaotropic agents, detergents.

#### Storage

Store at 4°C. Do not freeze.

#### Instructions for Use

- 1 Invert the column sharply several times to resuspend the settled gel and remove any bubbles.
- 2 Snap off the tip and place column in a 2.0 ml microcentrifuge tube (included). Remove cap. Allow the excess packing buffer to drain by gravity to top of gel bed. (If column does not begin to flow, push cap back into column and remove). Discard the drained buffer, then place the column back into 2 ml tube. However, if placing column into 12 x 75 mm test tube, centrifuge immediately.
- **3** Centrifuge for 2 min in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the packing buffer. Discard the buffer.
- **4** Place the column in a clean 2.0 ml microcentrifuge tube or 12 x 75 mm test tube. Carefully apply the sample (20–100 μl) directly to the center of the column. Application of more or less than the recommended sample volume may decrease column performance.
- **5** After loading sample, centrifuge the column for 4 min at 1,000 x g.
- **6** Following centrifugation, the purified sample is now in Tris or SSC buffer. Molecules smaller than the column's exclusion limit will be retained.
- 7 Properly dispose of the used column.

#### **Buffer Exchange**

The gel in the Bio-Spin columns is suspended in either SSC buffer, pH 7.0, or Tris buffer, pH 7.4. The gel matrix is compatible with most aqueous buffers. Buffer exchange can be achieved using the following procedure.



- 1 Follow steps 1, 2, and 3 in the Instructions for Use section.
- 2 Apply the new buffer in 500 µl aliquots. After each application of new buffer, let the buffer drain out by gravity, or centrifuge the column for 1 min to remove the buffer. Discard buffer from collection tube. Repeat as required. Three washes result in >99% of the buffer being exchanged. Four washes result in >99.9% of buffer exchanged.
- **s** Sample can now be applied to the column as directed in steps 4 through 7 in the Instructions for Use section.

#### **Centrifugation Notes**

Bio-Spin columns fit 2.0 ml microcentrifuge tubes or 12 x 75 mm test tubes for sample collection during centrifugation. Use the 2.0 ml microtubes provided with the columns for the initial column equilibration step.

Swinging bucket centrifuges capable of generating a minimum force of 1,000 x g are suitable for Bio-Spin column use. The gravitational force created at a particular revolution speed is a function of the radius of the microcentrifuge rotor. Consult the swinging bucket centrifuge instruction manual for conversion information from revolutions per minute (RPM) to centrifugal or g-force. Alternatively, to calculate the speed in RPM required to reach a gravitational force of 1,000 x g, use the following equation:

RCF (x g) =  $(1.12 \times 10^{-5}) \times (\text{RPM}) \times 2 \times \text{r}$ 

where RCF is the relative centrifugal force, r is the radius in centimeters measured from the center of the rotor to the middle of the Bio-Spin column, and RPM is the speed of the rotor.

#### Sterilization

If a sterile Bio-Spin column is required, autoclave the column at 121°C for 20–30 min. If exchanging buffers, the buffer pH in the column should be in the range of 6.0 to 8.0 prior to autoclaving.

#### **Ordering Information**

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Catalog #	Product Description
Bio-Spin Columns with Bio-Gel P-6 in Tris Buffer	
732-6227	Bio-Spin 6 Tris Columns, 25
732-6228	Bio-Spin 6 Tris Columns, 100
732-6229	Bio-Spin 6 Tris Columns, 1,000
Bio-Spin Columns with Bio-Gel P-30 in Tris Buffer	
732-6231	Bio-Spin 30 Tris Columns, 25
732-6232	Bio-Spin 30 Tris Columns, 100
732-6233	Bio-Spin 30 Tris Columns, 1,000
	umns with Bio-Gel P-6 in SSC Buffer
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732-6002	Bio-Spin 6 SSC Columns, 25
-	umns with Bio-Gel P-30 in SSC Buffer
732-6006	Bio-Spin 30 SSC Columns, 25
Micro Bio-Sp	in Columns with Bio-Gel P-6 in Tris Buffer
732-6221	Micro Bio-Spin 6 Tris Columns, 25
732-6222	Micro Bio-Spin 6 Tris Columns, 100
732-6225	Micro Bio-Spin 6 Tris Columns, 1,000
Micro Bio-Spin Columns with Bio-Gel P-30 in Tris Buffer	
732-6223	Micro Bio-Spin 30 Tris Columns, 25
732-6224	Micro Bio-Spin 30 Tris Columns, 100
732-6226	Micro Bio-Spin 30 Tris Columns, 1,000
-	in Columns with Bio-Gel P-6 in SSC Buffer
732-6200	Micro Bio-Spin 6 SSC Columns, 25
732-6201	Micro Bio-Spin 6 SSC Columns, 100
Micro Bio-Sp	in Columns with Bio-Gel P-30 in SSC Buffer
732-6202	Micro Bio-Spin 30 SSC Columns, 25
732-6203	Micro Bio-Spin 30 SSC Columns, 100
RNase-Free	Micro Bio-Spin Columns with Bio-Gel P-30 in Tris Buffer
732-6250	Micro Bio-Spin 30 Tris Columns, RNase free, 25
732-6251	Micro Bio-Spin 30 Tris Columns, RNase free, 100
Empty Colun	nns
732-6008	Empty Bio-Spin Columns, 100
732-6204	Empty Micro Bio-Spin Columns, 100
	n Mixture Purification
732-6300	PCR Kleen Spin Columns, 25
732-6301	PCR Kleen Spin Columns, 100
DNA Recover	ry from Agarose Gels
732-6165	Freeze-N-Squeeze Spin Columns, 25
732-6166	Freeze-N-Squeeze Spin Columns, 100
732-6160	Quantum Prep Gel Slice Kit
SEQueaky Kleen Terminator Removal Kit	
732-6260	SEQueaky Kleen, 2x96
732-6261	SEQueaky Kleen, 10x96



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