

Pierce[®] Concentrator

150K MWCO; 7mL and 20mL

89920 89921 89922 89923

2231.1

Number	Description
89920	Pierce Concentrator 150K MWCO, 7mL, 10 devices, for sample volumes up to 7mL
89921	Pierce Concentrator 150K MWCO, 20mL, 10 devices, for sample volumes up to 20mL
89922	Pierce Concentrator 150K MWCO, 7mL, 25 devices, for sample volumes up to 7mL
89923	Pierce Concentrator 150K MWCO, 20mL, 25 devices, for sample volumes up to 20mL

Molecular-weight cutoff (MWCO): 150,000

Storage: Upon receipt store at room temperature. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Concentrators are disposable ultrafiltration centrifugal devices for concentration and diafiltration/buffer exchange of biological samples such as enzymes, antibodies, DNA or virus particles. These concentrators provide exceptional protein recovery using an easy one-step procedure. The unique conical design and high-performance membranes maximize surface area while minimizing protein polarization and adsorption at the membrane surface, providing fast sample processing, high retentate recovery and high concentration factors (> 90 fold). The concentrators are compatible with most swinging-bucket (preferred) or fixed-angled rotors.

Important Product Information

- A centrifuge with swinging-bucket or fixed-angle rotor capable of holding 15mL or 50mL conical tubes. A minimum speed of $1000 \times g$ is required.
- The concentrators can be used effectively at a relative centrifugal force of $1000\text{--}4000 \times g$; the recommended speed is $2000 \times g$ for swinging-bucket and fixed-angle rotors. Do not exceed $2000 \times g$ for the 7mL concentrators. Do not exceed the maximum recommended centrifugal force of the rotor being used.
- Ensure rotor and carriers are compatible with centrifugal device and allow proper clearance for swinging-bucket rotors and/or centrifuge lid. Ensure devices and rotors are properly balanced before centrifugation.
- Sample volume, centrifugal force, temperature, sample concentration and viscosity affect filtration rate. Optimize centrifugal time for each application. Using a fixed-angle rotor at $2000 \times g$ and 22°C, 13.5mL of a 0.25mg/mL protein sample will typically decrease in volume by 20- to 30-fold in 15 minutes.
- The dead-stop volume for the 7mL device is approximately 10µL when processing samples < 6mL. The dead-stop volume for the 20mL device is approximately 20µL and 65µL in a fixed-angle and swinging-bucket rotor, respectively.

Note: Precipitation may occur at high concentration factors for some proteins. Maximum concentration factor is dependent on the specific protein, starting concentration and buffer system. Unless the stability of a protein has been determined, avoid concentrating to dead-stop. Pre-filling the collection tube with a known volume of buffer can control the dead-stop volume and final sample concentration factor (see Appendix A).

- The concentrators contain a high-performance regenerated cellulose membrane rated for retaining molecules greater than the MWCO. Reduced retentate recovery may occur below certain concentrations or with molecules that are less than the MWCO. Retentate recovery varies depending on the protein or starting concentration. See Appendix A for more information.
- Avoid scraping the membrane surface when adding or recovering sample.
- Do not autoclave concentrators.

Procedure for Sample Concentration

A. Pre-rinsing (Optional)

Note: The concentrator membranes contain glycerin as a humectant. Use this portion of the procedure only if trace amounts of glycerin are known to interfere with downstream analysis. For samples that do not require pre-rinsing, proceed to Section B.

1. Add 4mL (7mL concentrator) or 12mL (20mL concentrator) of ultrapure water or buffer to the upper sample chamber.
2. Cap and place concentrator assembly into rotor with proper counterbalance.
3. Centrifuge at appropriate centrifugal force until > 3mL (7mL concentrator) or > 5mL (20mL concentrator) of filtrate is produced. (see Important Product Information)
4. Shake device and collection tube to remove water/buffer.
5. Replace concentrator into tube and proceed directly to Section B.

Note: Do not allow membrane to dry or performance may be affected.

B. Sample Processing

1. Add sample to the upper chamber of the concentrator. For the 7mL concentrator, the maximum sample volume is 7mL using a swinging-bucket rotor and 4mL using a fixed-angle rotor. For the 20mL concentrator, the maximum sample volume is 20mL using a swinging-bucket rotor and 13.5mL using a fixed-angle rotor.

Note: To modify the dead-stop volume, add buffer to the collection tube as described in Important Product Information and Appendix A.

2. Cap and place concentrator assembly into rotor with proper counterbalance.
3. Centrifuge at appropriate centrifugal force until desired concentration factor is achieved.
4. Gently aspirate concentrated sample from upper chamber using an appropriate pipette tip for the sample volume.

Note: For best results, do not allow sample to remain in device for extended periods (i.e., > 15 minutes) after processing.

5. (Optional) For additional sample recovery add 50-100µL of buffer to the device and incubate for 10-15 minutes before recovering. Increase in recovery is generally < 5%. Confirm protein recovery amounts by protein assay (see Related Thermo Scientific Products) before combining wash with sample to avoid unnecessary dilution.

Procedure for Diafiltration/Buffer Exchange

1. Place sample into the upper sample chamber of the concentrator. For the 7mL concentrator, the maximum sample volume is 7mL using a swinging-bucket rotor and 4mL using a fixed-angle rotor. For the 20mL concentrator the maximum sample volume is 20mL using a swinging-bucket rotor and 13.5mL using a fixed-angle rotor.
2. Cap and place concentrator assembly into rotor with proper counterbalance.
3. Centrifuge at an appropriate centrifugal force until desired concentration factor is achieved. Dilute sample to original volume with Exchange Buffer. Repeat this step until desired solute removal has been achieved.

Troubleshooting

Problem	Possible Cause	Solution
Protein precipitation	Concentration is too high	Reduce concentration factor
	Solubility is poor in the buffer	Try an alternative buffer system to increase protein solubility
Low retentate recovery	Protein molecular weight is below the recommended cutoff	Use appropriate MWCO membrane
	Protein concentration is below the recommended concentration	Perform the sample concentration process twice
	Membrane is damaged – note that a damaged membrane may exhibit a slightly higher flux rate	Use a new concentrator and do not touch or scrape the membrane with the pipette tip
		Do not exceed recommended centrifugal force

Appendix

A. Dead-stop Control

Controlling the dead-stop volume will prevent over-concentrating proteins with known stability problems. The final sample volume placed in the upper sample chamber plus buffer in the collection tube can control the dead-stop volume. Sample concentration will cease when the volume in the collection tube rises to the meniscus in the sample chamber. The dead-stop volumes for various sample sizes are indicated in Tables 1 and 2. For example, 16mL of sample with 6mL of buffer placed in the collection tube (22mL total volume) will result in a dead-stop volume of ~200 μ L after centrifugation in a swinging-bucket rotor.

Table 1. Dead-stop volumes for various sample sizes using the 20mL concentrators.

Total Volume (sample + buffer in collection tube)		
<u>Swinging Bucket (mL)</u>	<u>35° Fixed Angle (mL)*</u>	<u>Dead Stop (μL)</u>
20.6	21.3	100
22.0	22.4	200
24.7	24.4	500
27.2	26.2	1000
30.2	28.5	2000

*Do not exceed the maximum recommend sample volume (13.5mL) for a fixed-angle rotor or sample loss may occur and rotor may become imbalanced.

Table 2. Dead-stop volumes for various sample sizes using the 7mL concentrators.

Total Volume (sample + buffer in collection tube)		
<u>Swinging Bucket (mL)</u>	<u>35° Fixed Angle (mL)*</u>	<u>Dead Stop (μL)</u>
< 6.0	< 5.0	$\leq$ 10
6.45	6.25	20
7.20	6.90	100
7.60	7.35	200
8.25	8.05	500
9.00	8.75	1000

*Do not exceed the maximum recommend sample volume (4mL) for a fixed-angle rotor or sample loss may occur and rotor may become imbalanced.

B. Chemical Compatibility

The regenerated cellulose membranes used in the Pierce Concentrators are compatible with most standard aqueous biological samples, buffers and salts (Table 3). Samples containing high levels of cell membranes, fats or lipids may reduce performance and result in membrane blockage.

Table 3. Concentrator chemical compatibility.*

<u>Acids and Bases</u>	<u>Rating</u>	<u>Organics</u>	<u>Rating</u>	<u>Miscellaneous</u>	<u>Rating</u>
Acetic acid (20%)	A	Acetone	NR	Glycerol	A
Hydrochloric acid 0.5M	A	Acetonitrile (20%)	A	Guanidine•HCl (6M)	A
Sodium hydroxide 0.5M	A	Benzene	NR	Polyethylene glycol	A
Trifluoroacetic acid (10%)	A	Chloroform	NR	Triton® X-100 (0.1%)	A
		Dimethyl sulfoxide (10%)	A	Tween®-20 (0.1%)	A
		Methanol (10%)	A	Urea (8M)	A
		Phenol	NR		

A = Acceptable **NR** = Not recommended

*Concentrations listed are provided as guidelines and do not necessarily represent maximum tolerances. Some compatible chemicals may modify the apparent molecular weight of molecules in the sample and/or the molecular-weight cutoff rating of the membrane.

C. Typical Performance

Protein recovery varies depending on protein starting concentration and molecular weight (Tables 4 and 5). Data in Tables 4 and 5 were generated using the 150K MWCO, 20mL concentrators with a 13.5mL starting volume and a fixed-angle rotor at $2000 \times g$ at 22°C. Performing the sample concentration procedure twice can increase the retentate recovery by 10% for protein concentrations ($< 0.25\text{mg/mL}$).

Table 4. Retention of various proteins using the 150K concentrators.

Protein	Concentration (mg/mL)	Retentate Recovery (%)
Ovalbumin (44kDa)	0.4	2
BSA (67kDa)	1	34
Conalbumin (78kDa)	0.5	37
Alcohol dehydrogenase (150kDa)	0.5	75
Aldolase (160kDa)	0.25	87

Table 5. Retention of rabbit IgG (150kDa) using the 150K concentrators.

Concentration (mg/mL)	Retentate Recovery (%)
0.1	60
0.25	84
0.5	93
1.0	100

Related Thermo Scientific Products

89884A	Pierce Concentrator 9K MWCO, 7mL, 25 devices
87748	Pierce Concentrator 9K MWCO, 7mL, 10 devices
89886A	Pierce Concentrator 20K MWCO, 7mL, 25 devices
87750	Pierce Concentrator 20K MWCO, 7mL, 10 devices
89885A	Pierce Concentrator 9K MWCO, 20mL, 25 devices
87749	Pierce Concentrator 9K MWCO, 20mL, 10 devices
89887A	Pierce Concentrator 20K MWCO, 20mL, 25 devices
87751	Pierce Concentrator 20K MWCO, 20mL, 10 devices
23225	Pierce BCA Protein Assay Kit
22662	Pierce 660nm Protein Assay Kit

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Current product instructions are available at www.thermoscientific.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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