

# iCON™ Concentrator

## 20 ml/9K and 20 ml/20K

89885 89887

1517.0

Number	Description
89885	iCON™ Concentrator 20 ml/9K, 25 devices, for sample volumes up to 20 ml Molecular weight cut-off (MWCO): 9,000
89887	iCON™ Concentrator 20 ml/20K, 25 devices, for sample volumes up to 20 ml Molecular weight cut-off (MWCO): 20,000

**Storage:** Upon receipt store at room temperature.

### Introduction

The iCON™ Concentrators are disposable ultrafiltration centrifugal devices for concentration and diafiltration/buffer exchange of biological samples such as enzymes, antibodies or DNA. These concentrators provide exceptional speed, protein recovery and ease-of-use in a 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 reliable and consistent results. Typical protein recovery for proteins larger than the membrane MWCO is >90% and greater than 150-fold concentration may be achieved in less than 45 minutes for the 9K MWCO and less than 15 minutes for the 20K MWCO. The iCON™ Concentrators are compatible with most swinging-bucket (preferred) or fixed-angled rotors.

### Important Product information

- The iCON™ Concentrators can be used effectively at a relative centrifugal force of 2,000-9,000 x g; the recommended speeds are 2,500-4,500 x g for swinging-bucket rotors and 5,000-7,000 x g for fixed-angle rotors.  
**Note:** The maximum centrifugal force for many swinging-bucket rotors is 3,000-4,000 x g. 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 6,000 x g and 22°C, 10 ml of a 0.2 mg/ml protein sample will typically decrease in volume by 20- to 30-fold in 25 minutes for the 9K MWCO or 10 minutes for the 20K MWCO.
- The dead-stop volume for the 20 ml devices is approximately 20 µl and 65 µl in a fixed-angle and swinging-bucket rotor, respectively, which allows 300-1,000-fold concentration factors.  
**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 concentration factor for a sample (see Appendix A).
- The concentrators contain a high-performance regenerated cellulose membrane rated for retaining molecules greater than the indicated MWCO (>90% recovery). Reduced recovery may occur with molecules that are less than the indicated MWCO. Recovery will vary depending on the specific protein or starting concentration.
- Avoid scraping the membrane surface when adding or recovering sample.
- Do not autoclave iCON™ Concentrators.

**Warranty:** Pierce products are warranted to meet stated product specifications and to conform to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to one year from date of sale for products used, handled and stored according to Pierce instructions. Pierce's sole liability for the product is limited to replacement of the product or refund of the purchase price. Pierce products are supplied for laboratory or manufacturing applications only. They are not intended for medicinal, diagnostic or therapeutic use. Pierce products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from Pierce Biotechnology. Pierce strives for 100% customer satisfaction. If you are not satisfied with the performance of a Pierce product, please contact Pierce or your local distributor.

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## Procedure for Sample Concentration using the 20 ml Devices

### A. Additional Materials Required

- Centrifuge with swinging-bucket or fixed-angle rotor capable of holding 50 ml conical tubes and a minimum speed of 2,000  $\times g$
- Pipette for final sample recovery (10-1,000  $\mu\text{l}$  tip)

### B. Pre-rinsing (Optional)

**Note:** The iCON™ Concentrator membranes contain glycerin as a stabilizer and storage aid. 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 C.

1. Add 12 ml 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 >5 ml of filtrate is produced. (see Important Product Information)
4. Shake device and collection tube to remove water.
5. Replace concentrator into tube and proceed directly to Section C.

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

### C. Sample Processing

1. Place sample into upper sample chamber of concentrator. Maximum sample volume is 20 ml using a swinging bucket rotor and 13.5 ml using a fixed angle rotor.

**Note:** To modify the dead-stop volume, add additional 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. Use a 200  $\mu\text{l}$  or similar pipette tip to gently aspirate concentrated sample from upper chamber.

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

5. (Optional) For additional sample recovery add 50-200  $\mu\text{l}$  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 Pierce Products) before combining wash with sample to avoid unnecessary dilution.

## Procedure for Diafiltration/Buffer Exchange

### A. Additional Materials Required

- Centrifuge with swinging-bucket or fixed-angle rotor for 50 ml conical tubes and rated for  $\geq 2,000 \times g$
- Pipette for final sample recovery (10-200  $\mu\text{l}$  tip)
- Exchange Buffer

### B. Sample Processing

1. Place sample into upper sample chamber of concentrator. Maximum sample volume is 20 ml using a swinging-bucket rotor and 13.5 ml 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.
4. Dilute sample to original volume with Exchange Buffer.
5. Repeat Steps 3 and 4 until desired solute removal have been achieved.

**Troubleshooting**

<b>Problem</b>	<b>Possible Cause</b>	<b>Solution</b>
Protein precipitation	Concentration too high	Reduce concentration factor
		Try an alternative buffer system to increase protein solubility
Low protein recovery	Protein molecular weight below recommended cut off	None
	Membrane damaged, protein in filtrate	Use a new concentrator and do not touch membrane with pipette tip
	<b>Note:</b> A damaged membrane may exhibit a slightly higher than expected flux rate.	Do not exceed recommended centrifugal force

**Appendix**

**A. Dead-stop Control**

Controlling the dead-stop volume will prevent overconcentrating 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 Table 1. For example, 16 ml of sample with 6 ml of buffer placed in the collection tube (22 ml total volume) will result in a dead-stop volume of ~200 µl after centrifugation in a swinging-bucket rotor.

**Table 1.** Dead-stop volumes for various sample sizes using a swinging-bucket or fixed-angle rotor.

<b>Total Volume (sample + buffer in collection tube)</b>		
<b>Swinging Bucket</b>	<b>Fixed Angle (35°)*</b>	<b>Dead Stop</b>
20.6 ml	21.3 ml	100 µl
22 ml	22.4 ml	200 µl
24.7 ml	24.4 ml	500 µl
27.2 ml	26.2 ml	1,000 µl
30.2 ml	28.5 ml	2,000 µl

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

**B. Chemical Compatibility**

The regenerated cellulose membranes used in iCON™ Concentrators are compatible with most standard aqueous biological samples, buffers and salts (Table 2). Samples containing high levels of cell membranes, fats or lipids may reduce performance and result in membrane blockage.

**Table 2.** iCON™ Concentrator chemical compatibility.\*

<b>Acids and Bases</b>	<b>Rating</b>	<b>Organics</b>	<b>Rating</b>	<b>Miscellaneous</b>	<b>Rating</b>
Acetic acid (20%)	A	Acetone	NR	Glycerol	A
Hydrochloric acid 0.5 M	A	Acetonitrile (20%)	A	Guanidine•HCl (6 M)	A
Sodium hydroxide 0.5 M	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 (8 M)	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 cut-off rating of the membrane.

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## Related Pierce Products

<b>89884</b>	<b>iCON™ Concentrator 7 ml/9K</b> , 25 devices
<b>89886</b>	<b>iCON™ Concentrator 7 ml/20K</b> , 25 devices
<b>89882</b>	<b>Zeba™ Desalt Spin Columns</b> , 25 columns, for 30-120 µl samples
<b>66830</b>	<b>Slide-A-Lyzer® Dialysis Cassette, 10K MWCO, 12-30 ml</b> , 6/pkg
<b>23225</b>	<b>BCA™ Protein Assay Kit</b> , sufficient to perform 500 standard tube assays

iCON™ Technology is protected by US Patent 6,269,957, and 6,357,601.

Slide-A-Lyzer® Dialysis Cassette Technology is protected by U.S. Patent # 5,503,741 and other patent pending.

BCA™ Technology is protected by U.S. Patent # 4,839,295.

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The most current versions of all product instructions are available at [www.piercenet.com](http://www.piercenet.com). For a faxed copy, contact customer service (in the USA call 800-874-3723) or your local distributor.

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