INSTRUCTIONS

iCON™ Concentrator 7 ml/9K and 7 ml/20K

PIERCE
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89884 89886

1516.0

Number	Description
89884	**iCON™ Concentrator 7 ml/9K**, 25 devices, for sample volumes up to 7 ml Molecular weight cut-off (MWCO): 9,000
89886	**iCON™ Concentrator 7 ml/20K**, 25 devices, for sample volumes up to 7 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 molecular weight cut-off is >90% and greater than 150-fold concentration may be achieved in less than 30 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, 4 ml of a 0.2 mg/ml protein sample will typically decrease in volume by 20- to 25- fold in 20 minutes for the 9K MWCO or 25- to 30-fold in 8 minutes for the 20K MWCO.

- The dead-stop volume of the 7 ml devices is approximately 10 µl when processing samples <6 ml or with decanting of excess filtrate from samples >6 ml, which allows for concentration factors in excess of 500-fold.

  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.

Procedure for Sample Concentration using the 7 ml Devices

A. Additional Materials Required

- Centrifuge with swinging-bucket or fixed-angle rotor for 15 ml conical tubes and rated for ≥2,000 *x g*
- Pipette for retentate (final sample) recovery (10-200 µl tip)

B. Pre-rinsing (Optional)

Note: The membranes within an iCON™ Concentrator 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 4 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 >3 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 7 ml using a swinging bucket rotor and approximately 4.5-5.0 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 µl 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 30-100 µl 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 15 ml conical tubes and rated for ≥ 2,000 *x g*
- Pipette for final sample recovery (10-200 µl tip)
- Exchange Buffer

B. Sample Processing

1. Place sample into upper sample chamber of concentrator. Maximum sample volume is 7 ml using a swinging-bucket rotor and 4.5-5.0 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

Problem	Possible Cause	Solution
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 **Note:** A damaged membrane may exhibit a slightly higher then expected flux rate.	Use a new concentrator and do not touch membrane with pipette tip
		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, 4 ml of sample with 3.2 ml of buffer placed in the collection tube (7.2 ml total volume) will result in a dead-stop volume of ~100 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.

Total Volume (sample + buffer in collection tube)		
Swinging Bucket	**Fixed Angle (35°)***	**Dead Stop**
<6.0 ml	<5.0 ml	≤10 µl
6.45 ml	6.25 ml	20 µl
7.20 ml	6.90 ml	100 µl
7.60 ml	7.35 ml	200 µl
8.25 ml	8.05 ml	500 µl
9.00 ml	8.75 ml	1,000 µl

*Do not exceed the maximum recommend sample volume (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™ Concentrators chemical compatibility.*

Acids and Bases	Rating	Organics	Rating	Miscellaneous	Rating
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 at apparent molecular weight of molecules in the sample and/or the molecular weight cut-off rating of the membrane.

Related Pierce Products

89885	**iCON™ Concentrator 20 ml/9K,** 25 devices
89887	**iCON™ Concentrator 20 ml/20K,** 25 devices
89882	**Zeba™ Desalt Spin Columns, 0.5 ml,** 25 columns, for 30-120 µl samples
66830	**Slide-A-Lyzer® Dialysis Cassette, 10K MWCO, 12-30 ml,** 6/pkg
23225	**BCA™ Protein Assay Kit,** sufficient to perform 500 standard tube assays

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