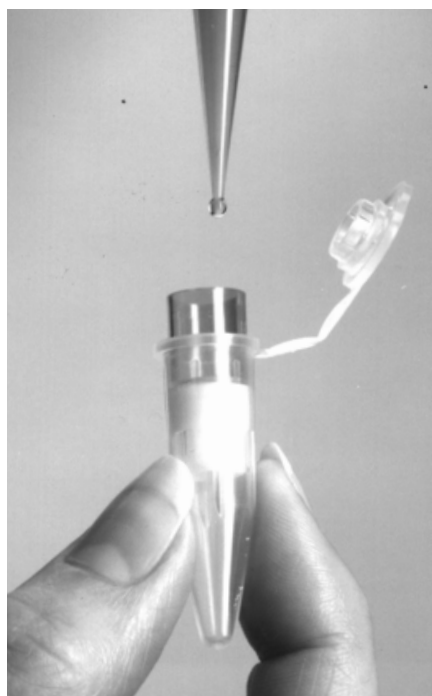


Microcon® Centrifugal Filter Devices

■ For up to 100X concentration of 50-500 μL with highest recovery



Microcon centrifugal filter devices offer a fast, convenient and reproducible means to concentrate, desalt, and purify microsamples of proteins and nucleic acids. Samples of up to 500 μL can be rapidly reduced to as little as 5 μL . These easy-to-use, disposable units incorporate a low-binding YM regenerated cellulose membrane and an inverted spin sample transfer method which delivers outstanding recoveries. A deadstop feature avoids risk of spinning your sample to dryness. A wide range of ultrafiltration cut-offs is offered. Microcon devices also provide excellent solvent resistance.

Microcon can be spun at up to 14,000 xg . Typical spin time (based on dead stop volume) for concentration at room temperature is 8 to 40 minutes, depending on cut-off used (100 minutes for Microcon YM-3).

Advantages

Compared to alternative methods and devices, Microcon offers essential benefits:

- Highest concentrate recovery
- Fast
- Highly reproducible performance
- No change in buffer composition during use
- Gentle, non-denaturing method
- No sample dilution when desalting (a potential problem with spin columns)
- No aerosols
- Minimal "hands-on" time compared with precipitation techniques

Features	Benefits
High Recovery <ul style="list-style-type: none"> • Inverted spin provides >98% recovery • Low-binding YM regenerated cellulose membrane • Low-binding materials of construction • Gentle, usable at 4 °C 	<ul style="list-style-type: none"> • Outstanding concentrate recoveries • High recovery of biological activity
Fast and Reproducible <ul style="list-style-type: none"> • Most separations completed in 8-40 min. • Reproducible results 	<ul style="list-style-type: none"> • Eliminates waiting for hours or overnight • Minimizes experimental variables
Convenient <ul style="list-style-type: none"> • Simple pipette and spin 	<ul style="list-style-type: none"> • Hands-on time is minimal
Versatile <ul style="list-style-type: none"> • Five molecular weight cut-offs • Scalable to large volume devices (Centricon, Centriplus) 	<ul style="list-style-type: none"> • Concentrate, desalt, remove primer, label, linkers, particles, etc., with one device • Further concentrate samples from larger devices
High Concentration Factors <ul style="list-style-type: none"> • 500 μL starting volume to 5 μL concentrate volume 	<ul style="list-style-type: none"> • 100 X concentration factors
Reliable <ul style="list-style-type: none"> • Disposable • Sample reservoirs color coded • Deadstop 	<ul style="list-style-type: none"> • No risk of cross-contamination • Avoid membrane cut-off mix-ups • Prevents sample loss

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■ Applications

Ethanol Precipitation Alternative

Ultrafiltration in Microcon devices provides a fast, efficient alternative to EtOH precipitation for recovery, concentration and desalting of nucleic acids. See Figure 1 for DNA recovery comparisons between the two methods. Figure 2 compares typical recoveries of nucleic acids with Microcon and by ethanol precipitation. ³²P labeled nucleic acid samples (pBR322, RNA transcript [pSPT 18-neo]) were spun in Microcon YM-30 at 7,000 xg for 5 min and a mixed base oligomer (25 mer) was spun in Microcon YM-3 for 1 hr. High salt concentration should be avoided with RNA and oligos, since this promotes binding of single-stranded nucleic acids to cellulose-based membranes. For EtOH precipitation, sodium acetate (0.3 M final concentration) and EtOH (2.5 vol) were added, and incubated overnight at -20 °C, then spun 20 min to collect the precipitate.

Concentration

Centrifugation of Microcon forces liquid through the low-binding membrane. Solutes larger than the nominal cut-off of the units are retained (select a cut-off about half of the MWCO of the desired concentrate). With dilute solutions, flow rates are directly proportional to g-force. At higher concentrations, increased viscosity and a polarizing layer at the membrane surface act to reduce the flow (as will low temperature). The design of Microcon reduces membrane polarization when spun at a fixed angle. The Amicon patented inverted-spin retentate transfer aids in highest sample concentrate recovery. Refer to Tables 2 and 3 for UF selection guidelines.

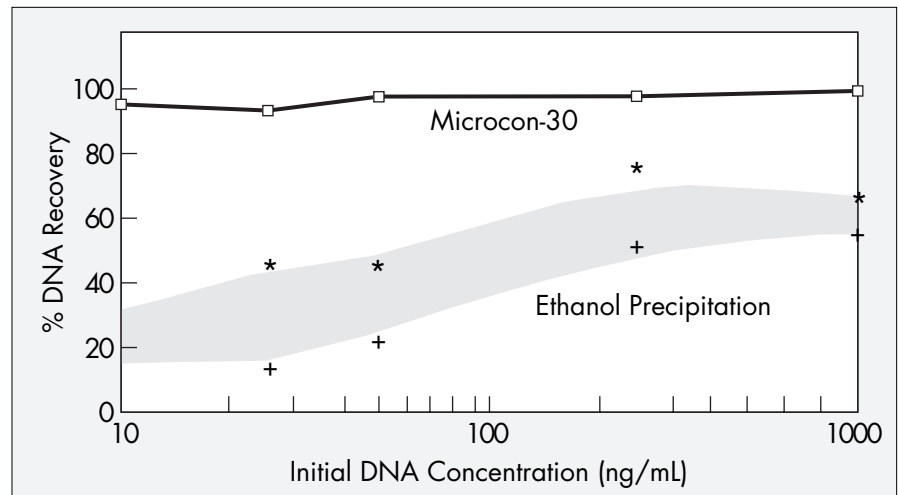


Figure 1. Comparison of DNA recovery with use of ethanol precipitation.

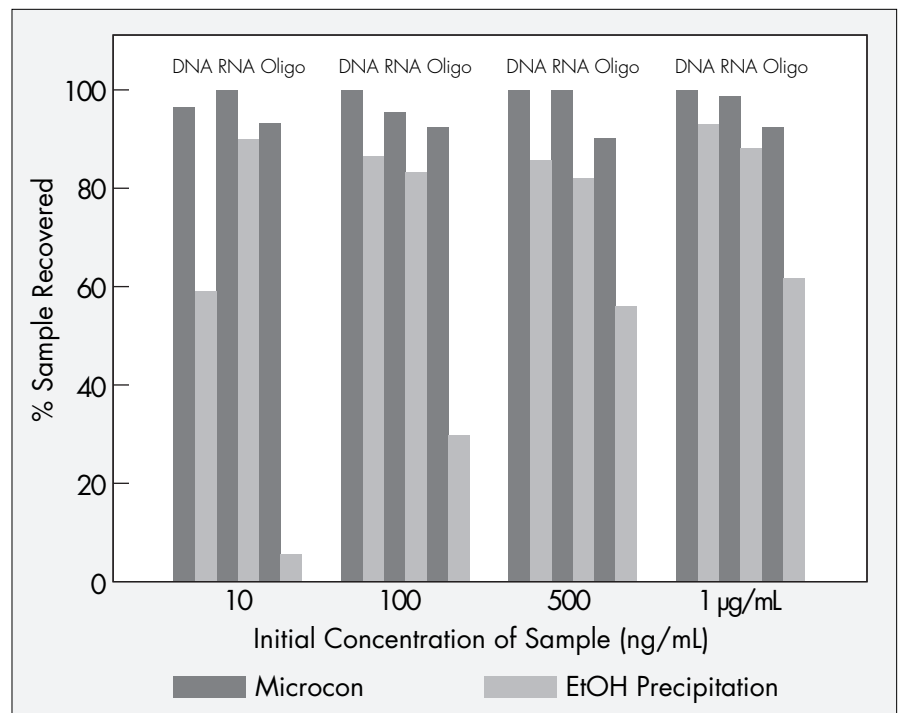


Figure 2. Nucleic acid recovery by Microcon and EtOH precipitation.

Desalting and Buffer Exchange

Microcon Centrifugal Filter Devices are a fast, convenient, high-recovery alternative to dialysis and ethanol precipitation. Sample dilution, often associated with spin columns, is not a problem. Salt transfer across the membrane is efficient and independent of microsolute concentration or size.

To desalt, concentrate the sample and reconstitute with buffer or water, then re-spin (this process is called diafiltration). Each cycle removes

90% of salt. Typically, three spins will remove 99% of salt. Very small volumes should be diluted to 500 μ L before spinning, possibly reducing salt to an acceptable level in a single run. (If a 100 μ L sample is diluted to 500 μ L, then concentrated to 5 μ L, 98% of salt will be removed).

Table 1 indicates typical diafiltration efficiency. Since Microcon YM-3 effectively removes riboflavin (370 MW), smaller buffer salt molecules can also be easily removed.

Purification

Microcon is effective in removing primers, linkers and unincorporated label from DNA or protein. Similarly, an analyte may be collected in a protein-free filtrate. Permeating molecules will pass at the same rate as salt because transport is independent of molecular size. Large proteins (>3,000 MW) may be separated from each other if they differ by a factor of ten in molecular size. As in desalting, small volumes should be pre-diluted to assist in purification. Refer to Tables 2 and 3 for UF selection guidelines.

Spin	Riboflavin Remaining	IgG Recovered
Initial	9%	95%
First Wash	2%	93%
Second Wash	<1%	93%
Third Wash	<1%	92%

Table 1. Removal of riboflavin and recovery of IgG. Microcon YM-3 filter devices containing 500 μ L of a 50:50 mixture of riboflavin/IgG were spun at 12,000 xg for 75 minutes at room temperature in 55° angle rotor. After the initial spin, the retentate was twice diluted with 500 μ L of PBS and spun again. After each spin, concentration of riboflavin and IgG in the filtrate and retentate were monitored. Riboflavin removal from and IgG recovery in the retentate are shown.

■ Selection Guide

Selecting the Cut-Off

The following tables contain membrane MW cut-off characteristics of each Microcon type and provide a guide to the selection of membrane type for specific applications. Table 2 includes nucleotide composition characteristics for use in DNA purification. Table 3 includes examples of typical recoveries with well characterized protein solutes.

Microcon Model	Color Code	Cut-off MW	Nucleotides		Max. g-Force	Spin Times		
			SS ¹	DS ¹		4 °C	25 °C	NC ²
3	Yellow	3,000	10	10	14,000	185	95	70
10	Green	10,000	30	20	14,000	50	35	20
30	Clear	30,000	60	50	14,000	20	12	10
50	Rose	50,000	125	100	14,000	10	6	6
100	Blue	100,000	300	125	DNA/RNA: 500	24	15	–

Table 2. Centrifugation and MW cut-off guidelines for nucleotides.

¹SS = single-stranded, DS = double-stranded.

²No Control.

■ Selection Guide, continued

Solute & Starting Concentration	Nominal MW	% Retentate recovery for Microcon by Model				
		3	10	30	50	100
Bovine IgG Fraction II (1 mg/ml)	156,000	95	95	95	95	95
Phosphorylase B (1 mg/ml)	97,400	65	65	70	80	80
Bovine serum albumin (1 mg/ml)	67,000	95	95	95	90	50
Ovalbumin (1 mg/ml)	45,000	95	95	95	90	10
α -Chymotrypsinogen (1 mg/ml)	25,000	95	95	95	80	2
Cytochrome c (0.25 mg/ml)	12,400	95	95	90	10	1
Protamine sulfate (1 mg/ml)	5,000-10,000	90	20	5	3	1
Vitamin B12 (0.2 mg/ml)	1,355	10	3	1	2	2
Riboflavin (saturated solution)	376	5	2	1	4	1

Table 3. Typical protein retentate recovery. 500 μ L samples reduced to 10 μ L. Time depends on solute composition, temperature, viscosity. Protein solutes: Microcon YM-3 and YM-10: Cytochrome c, 0.25 mg/mL; -30 and -50: BSA, 1 mg/mL; -100: IgG (Fraction II), 1 mg/mL.

■ Easy to Use

Three simple steps make operation very easy (see Figure 3). Hands-on time minimal. Use a microcentrifuge, preferably with fixed-angle rotor (a swinging-bucket centrifuge may also be used, but at lower g-force).

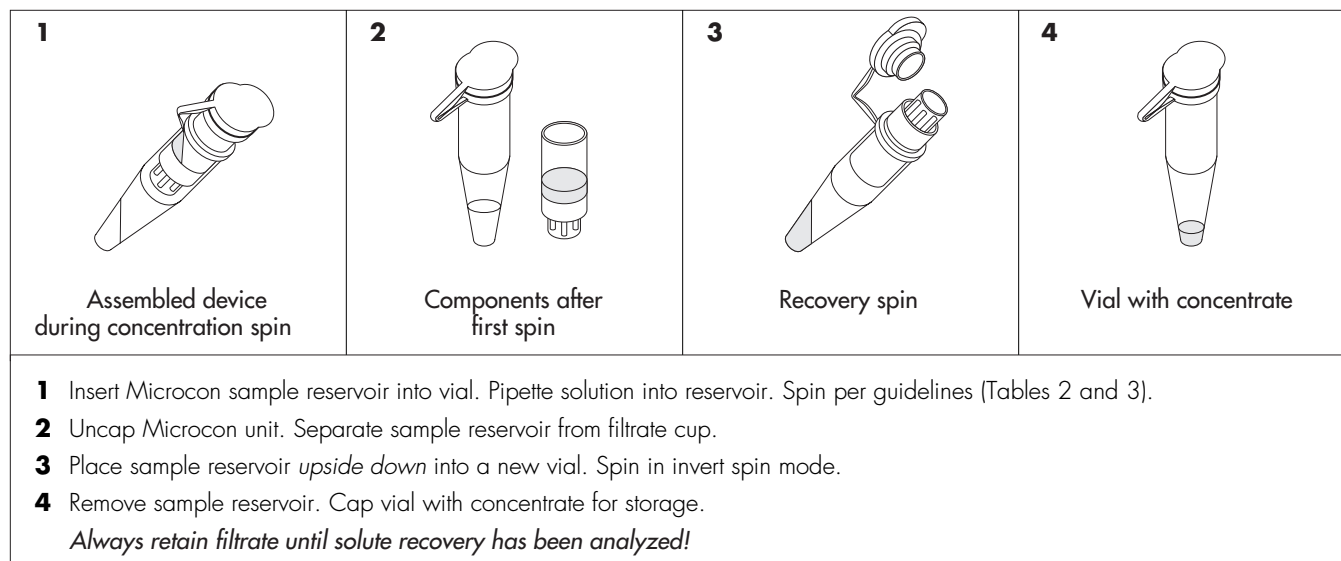


Figure 3. Operating Microcon microconcentrators.

■ Performance

Recovery

The anisotropic, hydrophilic YM ultra-filtration membranes in Microcon filter devices exhibit low levels of solute adsorption, leading to high sample recoveries. Recoveries of very dilute protein samples (less than 25 µg/mL

starting concentration) may require pretreatment of the Microcon device with passivation solutions.

Recoveries of nucleic acid samples (plasmids, vectors, restriction fragments, amplification reactions, oligos) are generally above 95%. For most

DNA samples of above 150 base pairs, Microcon-YM 100 provides an efficient and effective means of concentration and/or buffer exchange, without the need for ethanol precipitation. Refer to Figures 1 and 2 and to Table 2 for typical performance.

Rapid Results

Microcon filter devices provide exceptionally high flow rates. Filtration rates are affected by temperature (Figure 4) and starting volume (Figure 5).

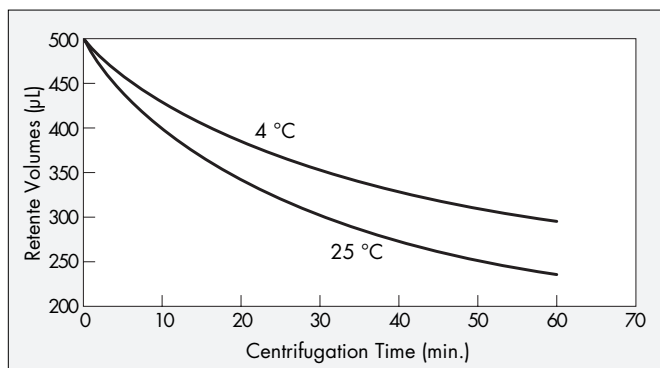


Figure 4. Effect of temperature on flow rate. Bovine serum with Microcon YM-30.

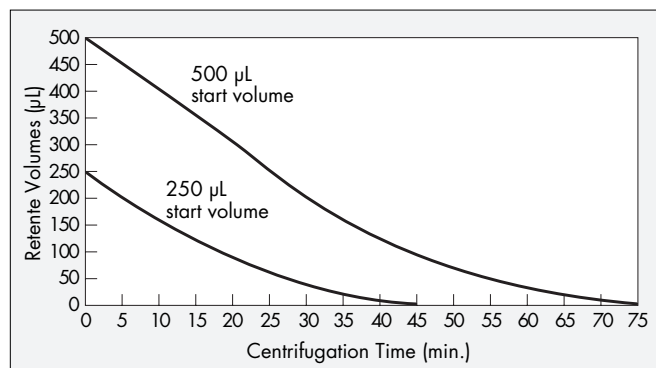


Figure 5. Effect of starting volume on flow rate. Microcon YM-3. 12,000 xg, room temperature, 55° angle rotor.

Chemical Resistance

Table 5 lists solutions that have been evaluated for chemical compatibility in Microcon devices in the 3 to 100,000 MW cut-off range.

Common Acids/Alkalis		Organic Solvents/Miscellaneous	
Acetic Acid (50%)	A	Acetone	NR
Ammonium Hydroxide (10%)	A	Acetonitrile (20%)	A
Formic Acid (50%)	A	Alconox (1%)	A
Formic Acid (70%)	NR	Ammonium Sulfate (saturated)	A
Hydrochloric Acid (1.0N)	A	Benzene	NR
Hydrochloric Acid (0.01N)	A	Carbon Tetrachloride	NR
Lactic Acid (50%)	A	CHAPS (100mM)	A
Sodium Hydroxide (0.1N)	A	Chloroform	NR
Sodium Hydroxide (2.5N)	NR	Diethyl Pyrocarbonate (0.2%)	A
Sulfuric Acid (3%)	A	Dimethyl Formamide	NR
Sulfuric Acid (30%)	NR	Dimethyl Sulfoxide (5%)	A
Trichloroacetic Acid (10%)	A	Dioxane	NR
Trifluoroacetic Acid (30%)	A	Ethyl Acetate	NR
A=Acceptable NR=Not Recommended		Formaldehyde (5%)	A
		Formamide	NR
		Glycerin	A
		Guanidine Thiocyanate (0.5M)	A
		Guanidine Thiocyanate (5M)	NR
		Guanidine HCl (6M)	A
		Lubrol PX (0.1%)	A
		Mercaptoethanol (0.1M)	A
Nonidet P-40 (2%)	A		
Phenol (1%)	A		
Phosphate Buffer (1M, pH 8.2)	A		
Polyethylene Glycol	A		
Pyridine	NR		
Sodium Carbonate (20%)	A		
Sodium Chloride (2M)	A		
Sodium Deoxycholate (5%)	A		
Terg-A-Zyme (1%)	A		
Tetrahydrofuran	NR		
Toluene	NR		
Tris Buffer (1M, pH 8.2)	A		
Triton Z-100 (5mM)	A		
Tween-20 (0.1%)	A		
Urea (8M)	A		

Table 4. Chemical Compatibility.

Specifications

Maximum initial sample volume:
500 µL

Typical final concentrate volume:
10-15 µL

Maximum relative centrifugal force:
14,000 xg

Active membrane area: 0.34 cm²

Materials of Construction

Membrane: High recovery
regenerated cellulose YM

Sample reservoir: Polycarbonate

Membrane support base: Acetal

Sample vial: Polypropylene

O-ring: Medical grade silicon rubber

Dimensions:

Diameter: 12.3 mm

Length

Concentration mode: 45.0 mm

Recovery mode: 48.2 mm

Ordering Information

Description	Catalogue No.
Microconcentrator and Cut-off	
Microcon YM-3	8/pk 42420
3,000 MW	24/pk 42403
(Yellow Base)	100/pk 42404
Microcon YM-10	8/pk 42421
10,000 MW	24/pk 42406
(Green Base)	100/pk 42407
Microcon YM-30	8/pk 42422
30,000 MW	24/pk 42409
(Clear Base)	100/pk 41410
Microcon YM-50	8/pk 42423
50,000 MW	24/pk 42415
(Rose Base)	100/pk 42416
Microcon YM-100	8/pk 42424
100,000 MW	24/pk 42412
(Blue Base)	100/pk 42413

With each order of Microcon devices, you receive sufficient numbers of sample vials to run each device.

To Place an Order or Receive Technical Assistance

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