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biotech

Directions for Use

Vivacell 70 | 100 | 250



85030-519-49

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# Vivacell 70

## Centrifugal Operation

The Vivacell 70 can be used in any centrifuge accepting 250 ml bottles. At just 1,000 xg and typically in less than 30 minutes up to 70 ml samples will be concentrated 50× and more, the built in dead stop impeding concentration to dryness. For large volumes and multiple samples four or more concentrators may be used, expanding volume capability to well over 250 ml.

## Pressurised Bench Top Operation

The Vivacell 70 can be pressurised and left to concentrate standing on a bench top, or in a refrigerator for maximum process control with minimum equipment requirements.

## Pressurised Operation on Laboratory Shaker

Single samples of up to 70 ml can be processed just as simply by using the Vivacell 70 Pressure Head and an orbital laboratory shaker. Assembly just takes a few seconds, whilst a single pressure charge is sufficient to reduce 50 ml to less than 2 ml in typically less than 20 minutes.

## Pressure-Fugation

Combining the advantages of both centrifugal and pressurised operation, Pressure-Fugation is recommended for viscous or difficult solutions such as serum, or when refrigeration is required which would otherwise slow the process. Samples of up to 50 ml can be processed, in typically less than 15 minutes.

## Equipment Required

### A. For use with Centrifuge

1. Centrifuge with swing bucket or fixed angle rotor (minimum 25°).
2. 250 ml centrifuge bottles for filtrate collection. (Prod No. VSA003). Bottles with modified caps are required for use in fixed angle rotors (Prod No: VCA004). Modified caps are supplied in the Vivacell 70 packs containing filtrate bottles.
3. Pasteur or fixed volume pipettes for sample recovery.

### B. For use with Gas Pressure

1. Vivacell 70 Pressure Head and 250 ml filtrate bottle (Product No. VCA700)
2. Air Pressure Controller (Product No. VCA002) or equivalent pressure regulator.
3. Pasteur or fixed volume pipettes for sample recovery.

### Optional Equipment

Laboratory orbital shaker accepting 250 ml bottles.

### C. For use with Pressure-Fuge

1. Vivacell 70 Pressure-fuge Head (Product No. VCA701).
2. Special Santoprene seals (Product No. VCA007) for Pressure-fuge Head
3. Charge Valve for Pressure-fuge Head (Product No. VCA005).
4. Air Pressure Controller (Product No. VCA002) or equivalent pressure regulator.
5. Centrifuge with swing bucket or fixed angle rotor (minimum 25°).
6. Pasteur or fixed volume pipettes for sample recovery.

## Operation

1. Select the most appropriate membrane cut-off for your sample. For maximum recovery select a MWCO at least 50% smaller than the molecular weight of your target molecule.
2. Place concentrator in filtrate bottle and fill with 10–70 ml (10–50 ml using Pressure-Fugation), of sample solution. Seal with Santoprene cap provided. In fixed angle rotors, modified bottle caps, (Prod No. VCA004), are also required. Modified caps are supplied in the Vivacell 70 packs containing filtrate bottles.

### A. Centrifuge

3. Insert assembled concentrator into centrifuge, in swing bucket rotors a specific device orientation is not necessary. In fixed angle rotors the device should be aligned with the concentration chamber window facing upwards | outwards.
4. Once the desired concentration is achieved (see table 3 for guide on concentration times), remove assembly and recover sample from the bottom of the concentrate pocket with a pipette.

### B. Using Gas Pressure

3. Attach Pressure Head (VCA700) to concentrator assembly and hand tighten to ensure an air tight seal.
- 4a. Set APC regulator to desired pressure (a pressure release valve factory set at 5 bar does not allow operation above this pressure). Lift regulator knob to unlock and turn in the appropriate direction (4 bar is recommended for most applications). Attach assembly to the female coupling to charge the Pressure Head. Detach from coupling by raising the outer locking sheath. One charge is normally sufficient to fully concentrate the sample.
- 4b. It is possible to use the concentrator assembly under constant pressure by attaching a female coupling (Prod. No. VCA010), to the extension line from the APC then attaching this female coupling to the Pressure Head as in 4a.
5. Leave to concentrate. For faster processing place on laboratory shaker and agitate gently at approximately 200–300 revs. a minute. Avoid high speed shaking as this is unnecessary and can cause foaming.
6. Once desired concentration level has been reached (see table 3 for guide on concentration times) remove assembly and de-pressurise by unscrewing Pressure Head or by releasing air from the centre of the inlet valve with a pointed instrument.
7. For maximum recovery, allow to stand after de-pressurisation for 10–15 minutes and then extract concentrate using a Pasteur or fixed volume pipette.

### C. Combining Gas Pressure with Centrifuge

3. Replace Santoprene cap with Santoprene seal supplied with Pressure-Fuge Head (VCA701) or order separately (VCA007). Attach Pressure-Fuge Head and hand tighten to ensure an air tight seal.
4. Attach Charge Valve (Prod No.VCA005) to APC extension line. After setting controller to desired pressure, as above, pressurise the concentrator assembly by pressing Charge Valve in to inlet valve on Pressure – Fuge Head. (Ensuring a gas tight seal).
5. Insert assembled concentrator into centrifuge and spin at maximum speeds shown in table 1.
6. Once desired concentration level has been reached, (see table 3 for guide on concentration times and spin speeds.) remove assembly and de-pressurise by unscrewing Pressure-Fuge Head.
7. Recover concentrate using a Pasteur or fixed volume pipette.

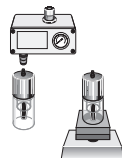
**Centrifuge**



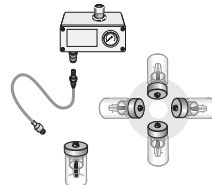
**Pressurise**



**Pressure Shake**



**Pressure Fuge**

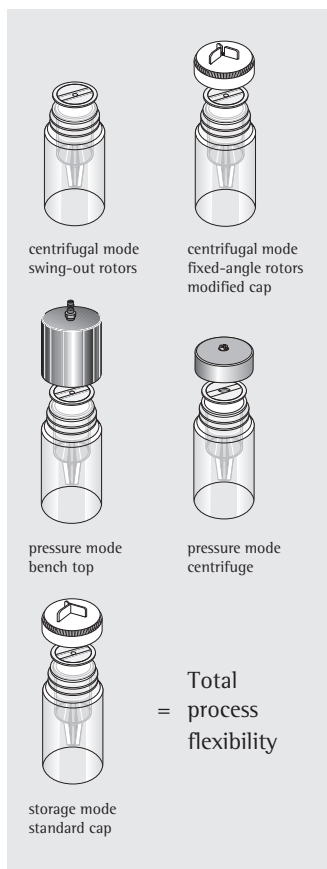


**Table 1: Recommended Spin Speed (x g)**

Mode	Centrifugal			Pressure-Fuge (5 bar max.)		
	min.	typical	max.	min.	typical	max.
Membrane						
5–50,000 MWCO PES	700	1,000	1,200	500	1,000	1,000
100,000 MWCO PES	700	1,000	1,200	500	1,000	1,000
PES 0.2 µm PES	700	1,000	1,200	500	1,000	1,000

**Table 2: Typical performance**

50 ml Start volume	Time to concentrate 30x min at 20°C				
	In centrifuge 1,000 g		As pressure cell 4 bar (60 psi) pressure		Solute recovery %
	No pressure	3 bar pressure	No agitation	Orbital shake	
<b>BSA 1.0 mg/ml (66,000 MW)</b>					
5,000 MWCO PES	37	18	50	25	96%
10,000 MWCO PES	25	15	45	20	96%
30,000 MWCO PES	22	13	45	20	93%
<b>IgG 0.25 mg/ml (160,000 MW)</b>					
50,000 MWCO PES	25	15	85	20	94%
100,000 MWCO PES	15	11	90	18	90%



**Table 3: Technical Specifications Vivacell 70**

**Concentrator capacity**

Swing bucket rotor	70 ml
Fixed angle rotor	50 ml
With pressure head	70 ml
With pressure-fuge head	50 ml

**Dimensions**

Total length	119 mm standard centrifugal 185 mm with pressure head 125 mm with pressure fuge head
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Width	62 mm
Active membrane area	20 cm <sup>2</sup>
Hold up volume of membrane	<200 µl
Dead stop volume	150 µl

**Operating requirements**

Rotor type	Swing bucket or fixed angle
Minimum rotor angle	25°
Rotor cavity	To fit 250 ml (62 mm) centrifuge bottles
Minimum effective speed	500 g
Recommended speed	1,000 g
Maximum speed	1,200 g
Maximum pressure	5 bar (75 psi)

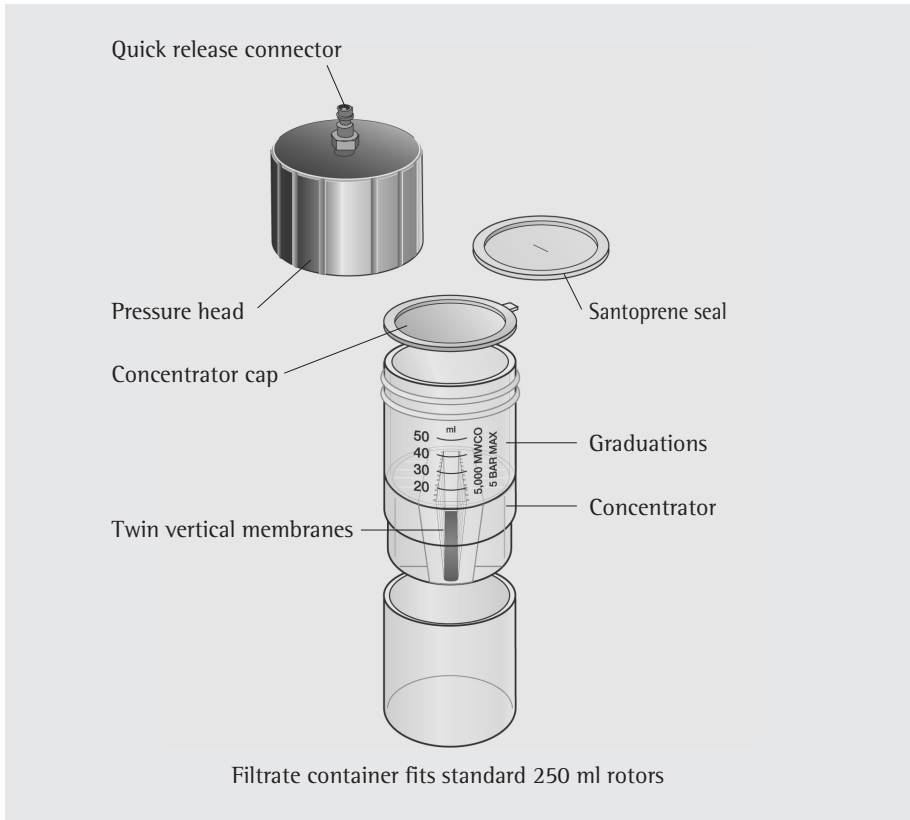
**Materials of construction**

Body	Polycarbonate
Filtrate vessel	Polycarbonate
Concentrator cap	Santoprene
Pressure head   pressure fuge head	Acetal
Membrane	Polyethersulfone

## Ordering information

<b>Vivacell 70 Polyethersulfone – concentrator bodies with polycarbonate filtrate bottles and modified caps</b>	<b>Pack size</b>	<b>Prod. no.</b>
5,000 MWCO	2	VS6011
10,000 MWCO	2	VS6001
30,000 MWCO	2	VS6021
50,000 MWCO	2	VS6031
100,000 MWCO	2	VS6041
0.2 µm	2	VS6071
<b>Vivacell 70 Polyethersulfone – concentrator body only</b>		
5,000 MWCO	10	VS6012
10,000 MWCO	10	VS6002
30,000 MWCO	10	VS6022
50,000 MWCO	10	VS6032
100,000 MWCO	10	VS6042
0.2 µm	10	VS6072
<b>Vivacell 70 accessories</b>		
Air pressure controller (APC) complete with pressure gauge, regulator, over-pressure safety valve, female connector to Sartorius Stedim Biotech pressure products and 1 m extension line (4 mm pneumatic tubing) with male and female connectors and 1 m of 6 mm inlet tubing	1	VCA002
250 ml centrifuge bottle – standard caps	4	VSA003
Modified caps for use in fixed angle rotors with 250 ml centrifuge bottles	2	VCA004
Charge valve for pressure-fuge head	1	VCA005
Replacement seals for pressure-fuge head (VCA701)	10	VCA007
Female connector	1	VCA010
Male connector	1	VCA011
4 mm pneumatic tubing (3 m)	1	VCA012
Vivacell 70 pressure head with reservoir and filtrate bottle (bench top use)	1	VCA700
Vivacell 70 pressure-fuge head (for use in centrifuge)	2	VCA701

## Vivacell 100

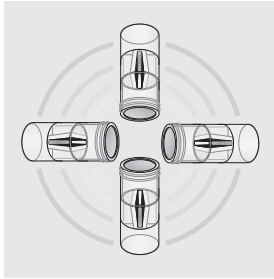


Like the smaller Vivacell 70 unit, Vivacell 100, when used as a centrifugal device, fits only into swing bucket rotors accepting 250 ml bottles. The Vivacell 100 can not be used in a fixed angle rotor.

Vivacell 100 units can also be used for single or extremely sensitive samples in the pressurized mode only and left on the bench or placed on a laboratory shaker for faster concentration. It can also be kept in a pressurized mode in the refrigerator. Handling is made easy by use of quick

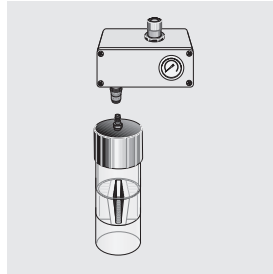
connectors. In whichever mode Vivacell 100 is used, the vertical membrane design inhibits membrane fouling while the built-in dead stop impedes concentration to dryness and loss of sample.





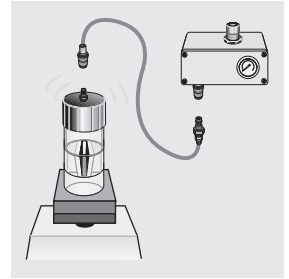
### Centrifuge

- Process convenience
- Low shear, no foaming
- Less visual control



### Pressure

- Simplicity and highest process control
- Ideal for refrigerated use
- Slower concentrations



### Pressure-shake

- Speed and process control
- Ideal for single samples

### Vivacell 100 Specifications

Without pressure head (Ø × L)	62 × 123 mm
With pressure head (Ø × L)	71 × 197 mm
Concentrator capacity	20–98 ml
Centrifuge rotor required	62 × 105 mm
Max centrifugal force	2,000 × g
Max pressure	5 bar (75 psi)
Active membrane area	23.5 cm <sup>2</sup>
Hold-up vol. memb. & support	< 250 µl
Dead stop volume	350 µl

### Vivacell 100 Materials of construction

Pressure head	Acetal
Quick release connector	Acetal
Concentrator cap (centrifugal)	Polypropylene
Concentrator   filtrate container	Polycarbonate
Pressure head seal	Santoprene

## Performance Characteristics

Typical performance 90 ml Start volume	Time to concentrate 30x min. at 20°C			Solute recovery %
	In centrifuge 2,000 g	As pressure cell 4 bar (58 psi) pressure		
	Swing out rotor	No agitation	Orbital shake	
<b>BSA 1.0 mg/ml (66,000 MW)</b>				
5,000 MWCO PES	22	75	25	96%
10,000 MWCO PES	16	60	20	96%
30,000 MWCO PES	16	60	20	94%
<b>IgG 0.25 mg/ml (160,000 MW)</b>				
50,000 MWCO PES	20	70	30	94%
100,000 MWCO PES	20	85	30	90%
<b>Latex beads 0.004% in DMEM + 10% FCS (0.055 µm)</b>				
300,000 MWCO PES	35	–	120	99%
<b>Latex beads 0.004% in DMEM + 10% FCS (0.24 µm)</b>				
1,000,000 MWCO <sup>†</sup> PES	4	5	4	99%

<sup>†</sup>2,000 g in centrifuge, 2 bar (29 psi) pressure

## Usage Tips

### 1. Flow Rate

Filtration rate is affected by several parameters, including membrane cut-off, sample concentration, viscosity, centrifugal force, pressure and temperature. Expect significantly longer spin times for starting solutions with over 5% solids. When operating at 4°C. flow rates are approximately 1.5 times slower than at 25°C. Viscous solutions such as 50% glycerine will take up to 5 times longer to concentrate than samples in a predominantly buffer solution.

### 2. Prerinsing

Membranes fitted to Vivacell concentrators contain trace amounts of Glycerine. Should this interfere with analysis this can be removed by rinsing approximately 50 ml of buffer solution or deionised water through the concentrator. Decant filtrate and concentrate before processing sample solution.

### 3. Sterilisation

Vivacell concentrators are not autoclaveable. To sterilise, use a 70% ethanol solution or sterilising gas mixture.

### 4. Desalting or Solvent exchange

The sample is first concentrated to desired level. Filtrate is discarded, then the device is refilled with an appropriate solvent. The sample is concentrated again and the process repeated until the concentration of the contaminating microsolutes is sufficiently reduced. Typically three wash cycles will remove 99% of initial salt content.

## 5. Cleaning and sanitising

Vivacell concentrators may be used several times if recommended cleaning and storage instructions are adhered to, and the membrane is not allowed to dry.

- I. Rinse out the device several times with deionized water.
- II. Fill the concentrator with a solution of 60% Ethanol and 40% 1M HCl. Alternatively fill concentrator with a dilute non ionic surfactant. Place the device in filtrate bottle and allow to soak for 1–2 hours. For best results, agitate on a laboratory shaker for approximately 30 minutes.

**⚠ Warning:** Strong alkaline solutions should not be used.

- III. Rinse thoroughly with clean water before re-use or storage.

## 6. Storage

Following cleaning Vivacell devices may be stored for several weeks in a 20% Ethanol solution. For best results, fill filtrate bottle with approximately 100 ml of solution, place concentrator in the bottle and then add a further 50 ml to the concentrator. This procedure will ensure that the membrane remains wetted throughout storage. Close with cap provided and preferably keep refrigerated.

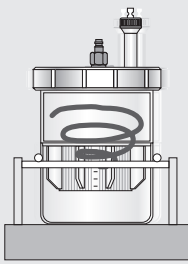
## Ordering information

<b>Vivacell 100 Polyethersulfone with Polypropylene concentrator cap</b>	<b>Pack size</b>	<b>Prod. no.</b>
5,000 MWCO	2	VC1011
5,000 MWCO	10	VC1012
10,000 MWCO	2	VC1001
10,000 MWCO	10	VC1002
30,000 MWCO	2	VC1021
30,000 MWCO	10	VC1022
50,000 MWCO	2	VC1031
50,000 MWCO	10	VC1032
100,000 MWCO	2	VC1041
100,000 MWCO	10	VC1042
300,000 MWCO	2	VC1051
300,000 MWCO	10	VC1052
1,000,000 MWCO	2	VC1061
1,000,000 MWCO	10	VC1062
0.2 µm	2	VC1071
0.2 µm	10	VC1072

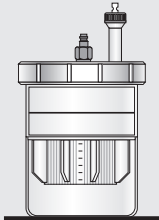
## Accessories

Air pressure controller (APC) complete with pressure gauge, regulator, over-pressure safety valve, female connector, 1 m extension line (4 mm pressure tubing) with male and female connectors and 1 m of 6 mm inlet tubing	1	VCA002
Plastic pipettes	100	VPA005
Female connector	1	VCA010
Male connector	1	VCA011
4 mm pressure tubing (3 m)	1	VCA012
Santoprene replacement seals	10	VCA014
Vivacell 100 pressure head with replacement seals (5)	1	VCA800

# Vivacell 250



Shake



Stand on bench



Recover

## Bench Top Operation

The Vivacell 250 can be used as a bench top concentrator. When used in conjunction with the Air Pressure Controller, (Prod. No. VCA002), the quick release inlet connector seals on dis-connection, allowing multiple devices to be charged with up to 4 bar pressure and left to concentrate. For even faster processing, single samples can be concentrated under constant pressure.

## Operation on Laboratory Shaker

Samples can be processed just as simply, and even faster by using an orbital laboratory shaker. Slow vortex action provides enhanced control of concentration polarisation, the constant swirling action reducing the build up of molecules on the membrane surface. Since only gentle motion is necessary the shearing effects typical of stirring mechanisms, are greatly reduced.

## Equipment Required

1. Air Pressure Controller (Prod. No.: VCA002) or equivalent pressure regulator.
2. Pasteur or fixed volume pipettes for sample delivery and recovery.

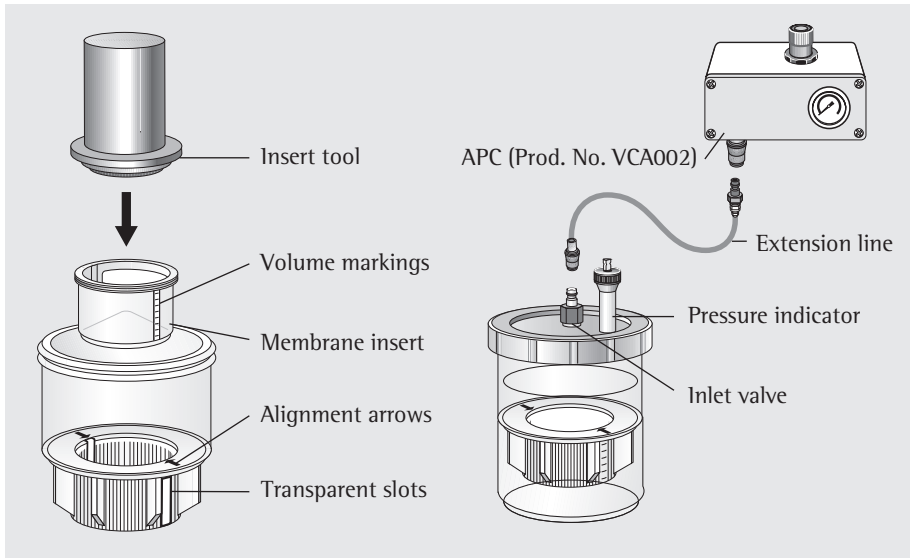
## Optional Equipment

Orbital Shaker accepting 1 litre bottles, (minimum stroke | orbit of 10 mm, minimum shaking frequency 170 RPM).

## Operation

### Assembly

1. Select the most appropriate membrane cut-off for your sample. For maximum recovery select a MWCO at least 50% smaller than the molecular size of your target molecule.
2. Line up the volume markings on the membrane insert with the transparent slots and alignment arrows in the sample reservoir and push down. Ensure the insert is fully seated by applying firm, even pressure with the insert tool. When installed correctly, the top of the insert should be level with the floor of the sample reservoir.



### Concentration

3. Fill with 50–250 ml, of sample solution.
4. Fit pressure head and hand tighten to ensure an air tight seal.
5. Using Air Pressure Controller (APC). Set controller to desired pressure by lifting regulator knob to unlock and turning in the appropriate direction (4 bar is recommended for most applications). Pressurise the concentrator assembly by connecting to the inlet valve on the pressure head.
  - a. To process single samples under constant pressure leave connected to APC extension line.
  - b. To charge multiple samples disconnect female connector by raising the outer locking sheath, and pressurise the next assembled device. One charge is normally sufficient to fully concentrate samples up to 150 ml. With larger volumes recharging may be necessary.

6. Leave to concentrate. For faster processing, place on laboratory shaker and agitate gently at approximately 200–300 rpm. Avoid high speed shaking, as this is unnecessary and can cause foaming. Leave to concentrate. (See usage tips and table 1. for guide on concentration times.)

### Concentrate Recovery

7. Once desired concentration level has been reached depressurise concentrator assembly by releasing air from the centre of the inlet valve with a pointed instrument.
8. For maximum recovery, allow to stand after de-pressurisation for 10–15 minutes and then extract concentrate using a Pasteur or fixed volume pipette.

### Insert Removal

Place the sample reservoir upside-down on a bench and push the insert down and out using the insert tool.

## Usage Tips

### 1. Flow Rate

Filtration rate is affected by several parameters, including membrane cut-off, sample concentration, viscosity, pressure and temperature. Expect significantly longer process times for starting solutions with over 5% solids. When operating at 4°C, flow rates are approximately 1.5 times slower than at 25°C. Viscous solutions such as 50% glycerine will take up to 5 times longer to concentrate than samples in a predominantly buffer solution.

### 2. Prerinse

Membranes fitted to Vivacell concentrators contain trace amounts of glycerine. Should this interfere with analysis this can be removed by placing approximately 200 ml of buffer solution or deionised water in the concentrator. Pressurising using one of the methods detailed above and allowing 100–150 ml to pass through the membrane. De-pressurise the device, then decant filtrate and concentrate before processing sample solution.

### 3. Sterilisation

Vivacell concentrators are not autoclaveable. To sterilise, use a 70% ethanol solution or sterilising gas mixture.

### 4. Chemical Compatibility

Vivacell concentrators are designed for use with biological fluids and aqueous solutions. For chemical compatibility, refer to table 3.

### 5. Desalting or Buffer Exchange

- I. Concentrate sample to desired level.
- II. Empty filtrate container.

III. Refill concentrator with an appropriate solvent.

IV. Concentrate the sample again and repeat the process until the concentration of contaminating microsolite is sufficiently reduced. Typically 3 wash cycles will remove 99% of initial salt content.

### 6. Cleaning and sanitising

Vivacell concentrators may be used several times if recommended cleaning and storage instructions are adhered to, and the membrane is not allowed to dry.

- I. Rinse out the device several times with deionized water.
- II. Fill the assembled concentrator with a solution of 60% ethanol and 40% 1M HCl. Alternatively, fill the concentrator with a dilute non ionic surfactant and allow to soak for 1–2 hours. For best results, agitate on a laboratory shaker for approximately 30 minutes.

**⚠ Warning:** Strong alkaline solutions should not be used.

III. Rinse thoroughly with clean water before re-use or storage.

### 7. Storage

Following cleaning, Vivacell membrane inserts may be stored for several weeks in a 20% ethanol solution. For best results, fill filtrate container or suitable beaker with solution, place membrane insert upright in the container. Ensure that the insert is fully submerged in the solution. This procedure will ensure that the membrane remains wetted throughout storage. Close with laboratory film and preferably keep refrigerated.

**Table 1: Technical Specifications Vivacell 250**

Concentrator capacity	250 ml
Max pressure	4 bar (60 psi)

**Dimensions**

Width	116 mm
Height (incl. pressure indicator)	235 mm
Active membrane area	40 cm <sup>2</sup>
Hold-up vol. memb. & support	<200 µl
Dead stop volume	600 µl

**Materials of construction**

Screw closure	Acetal
Pressure head	Acetal
Quick release connector	Acetal
Concentrator body   sleeve	Polycarbonate
Filtrate container	Polycarbonate

**Table 2: Typical performance**

	Time to concentrate 20x min at 20°C 4 bar pressure					
	100 ml start volume			250 ml start volume		
	Orbital shake	Free standing	Solute recovery %	Orbital shake	Free standing	Solute recovery %
<b>BSA 1.0 mg/ml (66,000 MW)</b>						
5,000 MWCO PES	19	70	98%	40	140	99%
10,000 MWCO PES	12	45	97%	28	100	98%
30,000 MWCO PES	12	45	96%	28	100	98%
<b>γ Globulins 0.25 mg/ml (160,000 MW)</b>						
30,000 MWCO PES	25	120	96%	55	240	98%
50,000 MWCO PES	25	120	94%	55	240	98%
100,000 MWCO PES	25	120	96%	58	240	98%



## Ordering information

<b>Vivacell 250</b>	<b>Pack size</b>	<b>Prod. no.</b>
Vivacell 250 complete with pressure head, pressure indicator   over-pressure release valve, quick release connection to APC, 2 sample reservoirs, filtrate container & insert tool	1	VCA250
<b>Vivacell 250 Polyethersulfone inserts</b>		
5,000 MWCO	5	VC2511
10,000 MWCO	5	VC2501
30,000 MWCO	5	VC2521
50,000 MWCO	5	VC2531
100,000 MWCO	5	VC2541
0.2 µm	5	VC2571
Starter kit (1 of each 5 k, 10 k, 30 k, 50 k, 100 k)	5	VC2551
<b>Accessories</b>		
Air pressure controller (APC) complete with pressure gauge, regulator, over-pressure safety valve, female connector to Sartorius Stedim Biotech pressure products and 1 m extension line (4 mm pneumatic tubing) with male and female connector and 1 m of 6 mm inlet tubing	1	VCA002
Replacement pressure indicator   over pressure relief valve	1	VCA008
Vivacell 250 maintenance kit (includes one sample reservoir and filtrate container, and "O" ring seals for pressure head)	1	VCA009
Female connector	1	VCA010
Male connector	1	VCA011
4 mm OD pressure tubing (3 m)	1	VCA012
Replacement pressure head & screw closure	1	VCA015

**Table 3: Chemical Compatibility VC 70-100-250**

<b>Solution</b>			
Acetic Acid (25.0%)	OK	Methanol (60%)	OK
Acetone (10.0%)	NO	Nitric Acid (10.0%)	OK
Acetonitrile (10.0%)	OK	Phenol (1.0%)	OK
Ammonium Hydroxide (5.0%)	?	Phosphate Buffer (1.0 M)	OK
Ammonium Sulphate (sat)	OK	Polyethylene Glycol	OK
Butanol (70%)	OK	Pyridine	NO
Chloroform (1.0%)	NO	Propanol 70%	OK
Dimethyl Formamide (10.0%)	NO	Sodium Carbonate (20%)	OK
Dimethyl Sulfoxide (5.0%)	OK	Sodium Deoxycholate (5.0%)	?
Ethanol (70.0%)	OK	Sodium Dodecylsulfate (0.01 M)	OK
Ethyl Acetate	NO	Sodium Hydroxide	NO
Formaldehyde (30%)	OK	Sodium Hypochlorite (200 PPM)	NO
Formic Acid (5.0%)	OK	Sodium Nitrate (1.0%)	OK
Guanidine HCl (6 M)	OK	Sulfamic Acid (3.0%)	OK
Hydrocarbons, aromatic	NO	Tetrahydrofuran (5.0%)	NO
Hydrocarbons, chlorinated	NO	Toluene (1.0%)	NO
Hydrochloric Acid (1 N)	OK	Trifluoroacetic Acid (10%)	OK
Imidazole (50 mM) (2 hr contact time)	NO	Tween 20 (0.1%)	OK
Lactic Acid (50.0%)	OK	Triton X-100 (0.001 M) (0.06%)	OK
Mercaptoethanol (10 mM)	OK	Urea (8 M)	OK

OK = Acceptable

? = Questionable

NO = Not recommended



Sartorius Stedim Biotech GmbH  
August-Spindler-Strasse 11  
37079 Goettingen, Germany

Phone +49.551.308.0

Fax +49.551.308.3289

[www.sartorius-stedim.com](http://www.sartorius-stedim.com)

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