

PROMETHEUS[®]

Protein Biology Products

Ultra-15 Centrifugal Filters



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Introduction

Ultra-15 Centrifugal Filters are designed for *in vitro* diagnosis, including analysis of concentrated serum, urine, cerebrospinal fluid and other body fluids. The vertical design and available membrane surface area provide fast sample processing, high sample recovery (> 90% sample recovery) and the capability for 80~100-fold concentration. The concentrate is collected from the filter device sample reservoir using a pipettor, while the ultrafiltrate is collected in the provided centrifuge tube. The filters can be spun in a swinging-bucket or fixed-angle rotor, are supplied non-sterile and are for **single-use only**.

Required Equipment

- Centrifuge with swinging-bucket or fixed-angle rotor with wells/ carriers that can accommodate 50ml tubes. **CAUTION:** To avoid damage to the device during centrifugation, check clearance before spinning.
- Pipettor with 200 microliter (μL) tip for concentrate recovery

Rinsing Before Use

Rinse the filters with buffer or water before use. If interference with analysis, rinse with 0.1N NaOH followed by a second spin of buffer or water.

CAUTION: Do not allow the membrane in the filters to dry out once wet. If you are not using the filters immediately after rinsing, leave fluid on the membrane until the device is used.

How to Use Ultra-15 Centrifugal Filters

1. Add up to 15ml of sample (12ml if using a fixed-angle rotor) to the filter device.
2. Place capped filter device into centrifuge rotor; counterbalance with a similar device.
3. When using a fixed-angle rotor, orient the device with the membrane panel facing up and spin at 5,000 x g maximum for 30 minutes. When using a swinging-bucket rotor spin at 4,000 x g maximum.
4. To recover the concentrated solute, insert a pipettor into the bottom of the filter device and withdraw the sample using a side-to-side sweeping motion to ensure total recovery. The ultrafiltrate can be stored in the centrifuge tube. **NOTE:** For optimal recovery, remove concentrated sample immediately after centrifugation.

Desalting or Diafiltration

Desalting, buffer exchange, or diafiltration are important methods for removing salts or solvents in solutions containing biomolecules. The removal of salts or the exchange of buffers can be accomplished in the filters by concentrating the sample, then reconstituting the concentrate to the original sample volume with any desired solvent. The process of “washing out” can be repeated until the concentration of the contaminating microsolute has been sufficiently reduced.

Protein Retention and Concentrate Recovery

The membranes used in the filters are characterized by a molecular weight cutoff (MWCO); that is, their ability to retain molecules above a specified molecular weight. Solutes with molecular weights close to the MWCO may be only partially retained. Membrane retention depends on the solute’s molecular size and shape. For most applications, molecular weight is a convenient parameter to use in assessing retention characteristics. For best results, use a membrane with a MWCO at least two times smaller than the molecular weight of the protein solute that one intends to concentrate.

Marker/ Concentration	Molecular Weight	MWCO	Min.	% Retention
BSA 1.0 mg/ml	67,000	30K	30	>95
BSA 1.0 mg/ml	67,000	50K	10	>90
Cytochrome c 0.25mg/ml	12,400	50K	10	<5
IgG 1.0 mg/ml	156,000	100K	20	>90
Ovalbumin 1.0 mg/ml	45,000	100K	20	<15

Factors that determine sample recovery include the nature of the protein solute relative to the device MWCO chosen, starting concentration and concentration factor.

Maximizing Sample Recovery

Low sample recovery in the concentrate may be due to adsorptive losses, over-concentration or passage of sample through the membrane.

- Adsorptive losses depend upon solute concentration, its hydrophobic nature, temperature and time of contact with filter device surfaces, sample composition and pH. To minimize losses, remove concentrated samples immediately after centrifugal spin.
- If the starting sample concentration is high, monitor the centrifugation process in order to avoid over-concentration of the sample. Over-concentration can lead to precipitation and potential sample loss.
- If the sample appears to be passing through the membrane, choose a lower MWCO filter.

Specifications

Concentrator Capacity

Swinging-bucket rotor 15ml

Fixed-angle rotor 12ml

Maximum Relative Centrifugal Force

Swinging-bucket rotor 4,000 × g

Fixed-angle rotor 5,000 × g

Dimensions - Filter Device in Tube (Capped)

Length: 117mm

Width: 29mm

Materials of Construction

Cap Polyethylene

Centrifuge tube Polypropylene

Filter MBS

Membrane PES

Other Specifications

Active membrane area 5.8cm²

Typical final concentrate volume 150 – 300µl