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1.0 Purpose

This procedure describes the use of Centrifugal Filter Devices (CFDs) for rapid and convenient desalting, buffer exchange and concentration of macromolecular solutions of 10 – 500 µL volumes. This will be used primarily as a sample preparation technique for assays in which a specific buffer or sample concentration may be necessary for analysis.

2.0 Scope

This procedure applies to Biopharmaceutical Process Analytics/Quality Control (PA/QC) personnel.

3.0 Authority and Responsibility

3.1 The Director of PA/QC has the authority to establish this procedure.

3.2 PA/QC personnel are responsible for the training of this procedure and documentation of training to Biopharmaceutical Quality Assurance (BQA).

3.3 PA/QC personnel are responsible for the performance of this procedure.

3.4 BQA is responsible for the quality oversight of this procedure.

4.0 Materials and Equipment

4.1 Centrifugal filter devices, BDP PN 22080, (Amicon Ultra 10K-0.5 CFD), BDP PN 31058, (Microcon 10K 0.5 ml CFD), or approved equivalent.

4.2 Any centrifuge capable of variable-speed settings accommodating 1.5 mL microcentrifuge tubes. Fixed-angle rotors are preferred.

4.3 (Optional) Exchange Buffer (exact buffer formulation will be specific for the product and assay).

4.4 High quality water (which includes deionized, reverse-osmosis, Milli-Q, WFI or other purified water).

5.0 Introduction

Centrifugal Filter Devices employ low-binding, anisotropic, hydrophilic regenerated cellulose membranes. The low-adsorption characteristics of the membrane and the device's component parts, together with an inverted recovery spin, combine to yield high recovery rates, typically >95% of the sample, with concentration factors as high as 100x. A full range of membrane cut-offs is available to perform a variety of functions, including concentration, desalting, buffer exchange, removal of primers, linkers or molecular labels from a reaction mix, and protein removal. Each filter device is supplied with 2 tubes. During operation, one tube is used to collect filtrate; the other to recover the concentrated sample. Maximum centrifugal force for the units is 14,000 x g. Excessive g-force may result in leakage or damage to the device. CFD components are not autoclavable and should be stored at room temperature.

6.0 Procedure

NOTE: If you are not familiar with the specific CFD, read the User Guide that comes with each package of CFDs. Ultra filtration membranes contain trace amounts of glycerin. If glycerin interferes with analysis, spin-rinse the unit with 0.5 mL of deionized water prior to use, according to the following procedure. Do not allow the membrane to completely dry before use.

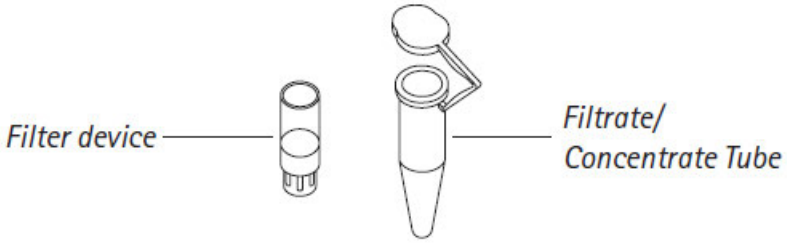

Figure 1. Millipore Microcon Filtration Device

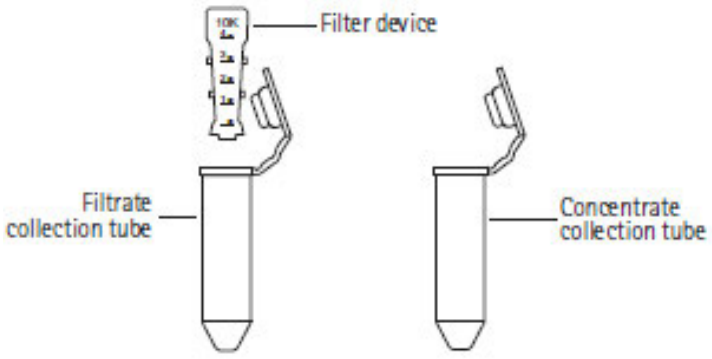

Figure 2. Millipore Amicon Ultra Filtration Device

6.1 Insert the filter device into a tube for filtrate collection.

6.2 Pipette the sample solution into the filtration device (0.5 mL maximum volume) without touching the membrane with the pipette tip. Microcon devices and Amicon Ultra devices have similar chemical compatibilities. Refer to Attachment I, Table 3 for a chemical compatibility list.

6.3 If the sample is to be buffer exchanged, add the volume of sample needed (10 µL minimum) and dilute up to a total volume of 0.5 mL with the desired exchange buffer. Seal the vial with the attached cap.

6.4 Place the assembly in a compatible centrifuge and counterbalance with a similar device. Align the cap strap toward the center of the rotor.

6.5 Centrifuge the sample(s) at 14,000 x g or according to the device User Guide (for example Attachment 1, Table 1). If performing a buffer exchange, add more buffer to the concentrated sample up to a total volume of 0.5 mL. This can be performed repeatedly, provided that the membrane is not spun down to dryness. Typically, two spins, each concentrating the sample 20-fold, will provide 95% exchange of buffers or removal of low-molecular-weight contaminants.

NOTE: Extended centrifugation can lead to dryness of the membrane. If this should occur, add at least 10 µL of buffer to the sample reservoir, agitate gently for 30 seconds, and continue with the procedure. Keep in mind that this can potentially result in sample loss or low sample recovery.

6.6 Remove the assembly from the centrifuge, uncap the vial, and separate the filtration device from the filtrate tube.

6.7 Place a new tube over the top of the device. Invert the assembly and centrifuge for 3 minutes at 1000 x g (or pulse briefly) to transfer the sample concentrate to the collection tube.

6.8 Remove the assembly from the centrifuge, discard the filtration device in a suitable waste container, close the collection tube cap and store the sample at the desired temperature for later use. Refer to Attachment I, Table 2 for a list of typical protein recoveries for each Microcon model.

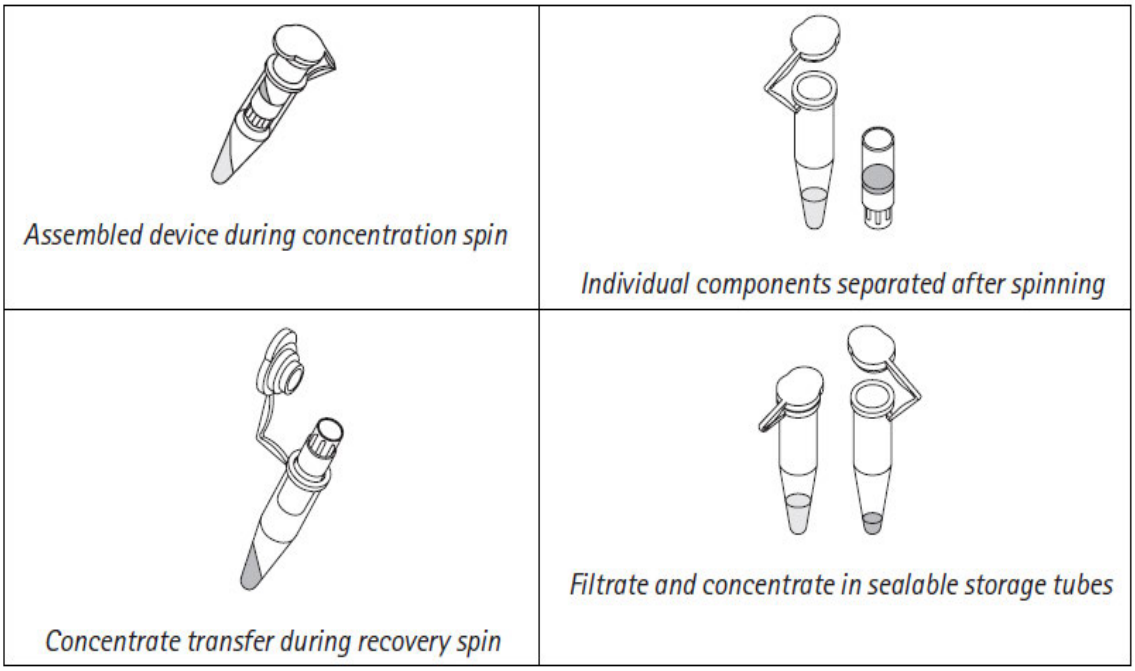

Figure 3. Sample Diagrams

7.0 Documentation

7.1 Document the UFD model number, lot number, and expiration date, the sample information, and the exchange buffer formulation (if applicable), lot number and expiration date, in an issued laboratory notebook or other controlled document.

8.0 Attachments

8.1 **Attachment 1** Centrifugation Guidelines (Table 1),
Typical Protein Recoveries (Table 2),
Chemical Compatibility (Table 3)

[redacted]

Attachment 1

Table 1: Centrifugation Guidelines - Typical Spin Times and Concentration Factors

Application	Device	G-force (x g)	Typical Spin Time (minutes)	Target Concentration Factor
Protein	10K	14,000	20–40	20–100
Protein	30K	14,000	10–20	20–100
DNA	DNA Fast Flow	500	10–20	≤ 20

* These guidelines are for starting volumes of 500 µL. For starting volumes less than 500 µL, spin times will be shorter.

Table 2: Typical Recoveries of Proteins

Solute/Concentration	Molecular Weight	Typical % Recovery from Concentrate	
		10K Device	30K Device
Bovine IgG Fraction II (1 mg/mL)	156,000	95	95
Bovine serum albumin (1 mg/mL)	67,000	95	95
Ovalbumin (1 mg/mL)	45,000	95	95
α-Chymotrypsinogen (1 mg/mL)	25,000	95	95
Cytochrome c (0.25 mg/mL)	12,400	95	90
Protamine sulfate (1 mg/mL)	5,000–10,000	20	5
Vitamin B12 (0.2 mg/mL)	1,355	3	1
Riboflavin (saturated solution)	376	2	1

Attachment I (continued)

Table 3: Chemical Compatibility List

Acids	Concentration		Concentration
Acetic acid	≤ 50%*	Phosphoric acid	≤ 30%
Formic acid	≤ 5%*	Sulfamic acid	≤ 3%
Hydrochloric acid	≤ 1.0 M	Sulfuric acid	≤ 3%
Lactic acid	≤ 50%	Trichloroacetic acid (TCA)	≤ 10%*
Nitric acid	≤ 10%	Trifluoroacetic acid (TFA)	≤ 30%*
Alkalis			
Ammonium hydroxide	≤ 10%	Sodium hydroxide	≤ 0.5 M
Alcohols			
n-Butanol	≤ 70%	Isopropanol	≤ 70%
Ethanol	≤ 70%	Methanol	≤ 60%
Detergents			
Alconox® detergent	≤ 1%	Lubrol® PX detergent	≤ 0.1%
CHAPS detergent	≤ 0.1%	Nonidet™ P-40 surfactant	≤ 2%
Sodium deoxycholate	≤ 5%	Triton® X-100 surfactant	≤ 0.1%
Sodium dodecyl sulfate (SDS)	≤ 0.1%	Tween® 20 surfactant	≤ 0.1%
Tergazyme® detergent	≤ 1%		
Organic solvents			
Acetone	not recommended	Ethyl acetate	not recommended
Acetonitrile	≤ 20%	Formaldehyde	≤ 5%
Benzene	not recommended	Pyridine	not recommended
Carbon tetrachloride	not recommended	Tetrahydrofuran	not recommended
Chloroform	not recommended	Toluene	not recommended
Dimethyl sulfoxide (DMSO)	≤ 5%*		
Miscellaneous			
Ammonium sulfate	Saturated	Phenol	≤ 1%
Diethyl pyrocarbonate	≤ 0.2%	Phosphate buffer (pH 8.2)	≤ 1 M
Dithiothreitol (DTT)	≤ 0.1 M	Polyethylene glycol	≤ 10%
Glycerine	≤ 70%	Sodium carbonate	≤ 20%
Guanidine HCl	≤ 6 M	Tris buffer (pH 8.2)	≤ 1 M
Imidazole	≤ 100 mM	Urea	≤ 8 M
Mercaptoethanol	≤ 0.1 M		

* Contact with this chemical may cause materials to leach out of the component parts. Solvent blanks are recommended to determine whether leachables represent potential assay interferences.