Detergent removal using Amicon® Ultra or Microcon® centrifugal ultrafilters

Introduction
Microcon® and Amicon® Ultra centrifugal filters are efficient laboratory tools for removing small molecules from solutions of proteins or nucleic acids. Often, the molecule to be removed is one of a number of commonly used detergents or protein solubilizing agents. The chemical nature of most detergents allows for micelle formation above a critical concentration limit (Critical Micelle Concentration, CMC). Micelle formation results in aggregation of the detergent and leads to gross changes in molecular structure. This affects the amount of the detergent that can be removed from a solution by centrifugal devices with specific nominal molecular weight limit (NMWL) membranes.

For example, the monomer of Triton® X-100 has a molecular weight of 500–650 daltons. Triton® X-100 should pass readily through the 10,000 NMWL membrane in an Amicon® Ultra device. However, at concentrations above 0.01% (0.2 mM), Triton® X-100 forms micelles composed of approximately 140 monomeric units. During ultrafiltration, the micelles behave like 70,000–90,000 dalton globular proteins.

As a result, more than 90% of Triton® is retained by the ultra-filtration membrane. Therefore, above the CMC of Triton® X-100, an Amicon® Ultra-4 100K NMWL concentrator would be required to remove the detergent effectively.

Method and Results
In a series of studies, Merck Millipore researchers used Total Organic Carbon (TOC) analysis to measure detergent removal by Amicon® Ultra or Microcon® concentrators after a single centrifugation spin (note that complete detergent removal generally requires 3–5 spins). As the results in the Tables 1.1 and 1.2 indicate, detergent removal depends both on the original detergent concentration and the NMWL of the centrifugal units. All measurements shown were made using detergent/distilled water solutions.

NOTE: Temperature, the presence of salts in the solution, and/or macromolecule/detergent interactions may lower the CMC for a particular detergent. Use the tables only as general guidelines in assessing the efficiency of detergent removal with the Amicon® Ultra and Microcon® devices.

Table 1.1 Percent detergent removal after one spin with Amicon® Ultra-4 centrifugal devices

NMWL		
Detergent	10 kDa	30 kDa
SDS		
0.1%	95%	98%
1.0%	38%	48%
5.0%	94%	95%
Tween®-20		
0.1%	30%	42%
1.0%	28%	35%
5.0%	82%	77%
Triton® X-100		
0.1%	3%	47%
1.0%	2%	20%
5.0%	2%	20%
CHAPS		
0.1%	90%	96%
1.0%	66%	93%
5.0%	38%	73%

Table 1.2 Percent detergent removal after one spin with Microcon® centrifugal devices

NMWL					
Detergent	3 kDa	10 kDa	30 kDa	50 kDa	100 kDa
SDS					
0.01%	>90%	>90%	>90%	>90%	>90%
0.1%	>90%	>90%	>90%	>90%	>90%
1%	40-89%	40-89%	40-89%	40-89%	40-89%
5%	<40%	<40%	<40%	40-89%	40-89%
Na Deoxycholate					
0.1%	>90%	>90%	>90%	>90%	>90%
1%	>90%	>90%	>90%	>90%	>90%
5%	40-89%	40-89%	40-89%	>90%	>90%
CAPS					
5%	>90%	>90%	>90%	>90%	>90%
CPCL[1]					
0.01%	>90%	>90%	>90%	>90%	>90%
0.1%	40-89%	40-89%	40-89%	40-89%	40-89%
1%	<40%	<40%	<40%	<40%	40-89%
5%	<40%	<40%	<40%	<40%	40-89%
TDMABr[2]					
0.1%	>90%	>90%	>90%	>90%	>90%
1%	<40%	<40%	<40%	40-89%	>90%
5%	<40%	<40%	<40%	<40%	>90%
Digitonin					
0.01%	>90%	>90%	>90%	>90%	>90%
0.1%	40-89%	40-89%	40-89%	40-89%	40-89%
1%	<40%	<40%	<40%	<40%	<40%
Tween®-20					
0.01%	<40%	<40%	40-89%	40-89%	>90%
0.1%	<40%	<40%	<40%	40-89%	>90%
1%	<40%	<40%	<40%	<40%	40-89%
5%	<40%	<40%	<40%	<40%	<40%
Triton® X-10					
0.01%	40-89%	40-89%	40-89%	40-89%	>90%
0.10%	<40%	<40%	<40%	<40%	>90%
1%	<40%	<40%	<40%	<40%	40-89%
5%	<40%	<40%	<40%	<40%	<40%
CHAPS					
0.10%	>90%	>90%	>90%	>90%	>90%
1%	40-89%	40-89%	>90%	>90%	>90%
5%	<40%	<40%	40-89%	>90%	>90%

[1]Cetylpyridinium chloride
[2]Tetradecyltrimethylammonium bromide