# Protein and DNA Transmission Using Acrodisc® Syringe Filters

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## Introduction

Biopharmaceutical solutions from R&D to manufacturing are precious commodities. Often they are heat labile and diltue, leaving cold filtration the primary method for sterilization. Some concerns have been raised about protein loss with filtration. Successful transmission of these critical components is crucial to the development of process-scale handling in the biopharmaceutical industry. Integrating Pall's UpScale$^{SM}$ program into the drug development process eliminates the need to re-evaluate filtration systems at later stages of development or manufacturing.

Acrodisc syringe filters that are part of the UpScale program are available with a selection of four hydrophilic membranes: Fluorodyne® II (PVDF), Supor® (PES), Ultipor® (nylon), and Posidyne® (positively-charged nylon) membranes (Table 1). In this paper we demonstrate the use of Acrodisc syringe filters for the filtration of biomolecule-containing solutions without the loss of critical components. In addition, we developed passivation techniques to support the use of our chemically resistant Ultipor (nylon) membrane.

**Table 1**
Membrane Descriptions

| Membrane Name | Filter Media | Characteristics |
|---|---|---|
| Fluorodyne II | Hydrophilic Polyvinylidene | For high flow Fluoride rates and low protein binding |
| Supor | Hydrophilic Polyethersulfone | For high throughput, fast filtration, and low protein binding |
| Ultipor | Uncharged Nylon 6,6 | For broad solvent and chemical compatibility, and low extractables |
| Posidyne | Positively-charged Nylon 6,6 | For enhanced bioburden and pyrogen removal from aqueous solutions |

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## Protein Transmission

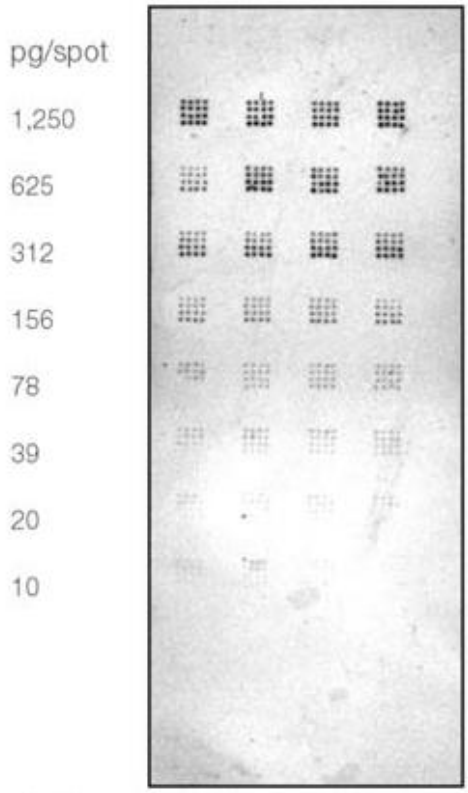
Protein transmission is the filtration of a solution without significant loss of critical components. Historically, membrane transmission studies have been performed under static soak conditions rather than actual filtration conditions. Static-soak test methods measuring protein transmission simply quantifies the saturation kinetics of the membrane rather than the binding rate of the membrane. Regardless of the static-soak binding kinetics, biomolecule loss during the act of filtration is the primary concern for the researcher. See Filtration of Dilute Protein and DNA Solutions.

To directly compare protein transmission during filtration, each Acrodisc syringe filter was tested using two different concentrations of dilute radioactive iodine-labeled bovine serum albumin (BSA) (ICN Biochemical). The sterile Acrodisc filters containing Fluorodyne II, Supor, Ultipor, and Posidyne membranes were challenged with two different 5 mL samples of $^{125}$I-labeled BSA solutions in 1x PBS at a total concentration of 0.25 µg and 1.25 µg BSA respectively (Figure 1). The resulting filtrate was collected and a 100 µL sample was counted using a liquid scintillation multilabel counter (PerkinElmer Wallac,

Gaithersburg, MD, USA).

Fluorodyne II and Supor membranes had nearly 100% protein transmission of the BSA solution at both concentrations (Figure 1). Ultipor membrane showed a reduction in protein transmission when compared to Fluorodyne II and Supor, and the Posidyne membrane (positively-charged nylon used to enhance bioburden and pyrogen removal from aqueous solutions) had the lowest transmission of all four membranes. The Ultipor membrane is chemically resistant and has a long history in the pharmaceutical industry. To increase protein transmission using the Ultipor membrane, a passivation step can be used.

**Figure 1**
Dilute Protein Transmission Through Select 25 mm Devices




*Dilute protein solutions of both 50 ng/mL and 250 ng/mL were filtered using separate 10 mL syringe fiters at 1 mL/sec. Each 5 mL sample contained 0.25 µg and 1.25 µg $^{125}$I BSA respectively. The filtrate for each filter was collected; 100 µL samples were pipetted into separate scintillation vials and counted in a multilabel counter (PerkinElmer Wallac, Gaithersburg, MD, USA). The data was normalized against the absolute values measured from individual membrane sample counts (see Filtration of Dilute Protein and DNA Solutions) as well as unfiltered controls sampled at the beginning and end of each sampling. Error bars indicate standard deviation (n = 30 Acrodisc syringe filters).*

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## Use of Passivation to Increase Protein Transmission

Passivation involves the filtration of another pretreatment solution to block binding sites prior to the filtration of solutions containing critical components. The Ultipor membrane was passivated with a variety of soltuions before the filtration of dilute $^{125}$I-labeled BSA. Three different passivation conditions were tested: 1% BSA, 5% sodium dodecyle sulfate (SDS), and 5% Tween 80. For consistency, two lots of each Acrodisc syringe filter type, five samples per lot, were tested under each passivation condition. To passivate protein binding, 1 mL of passivation solution was filtered through the Acrodisc device, followed by the filtration of the dilute iodine-labeled BSA solution (150 ng/mL).

Subsequent analysis of the transmitted $^{125}$I-labeled BSA indicated that some passivation occurred with each of the three solutions tested (Figure 2). The use of 5% Tween 80 allowed the greatest transmission of the iodine-labeled BSA challenge solution and is recommended for optimal recovery when using the Ultipor membrane.

**Figure 2**
Passivation Techniques Can Improve Protein Transmission
Profiles for Acrodisc Syringe Filters with Ultipor Membrane

*Three different passivation solutions, 1% BSA, 5% SDS, and 5% Tween 80, were tested for their ability to reduce biomolecule binding on two lots of five samples for each Acrodisc syringe filter. First, 1 mL of the respective passivation solution was passed through the membrane and discarded, followed by the filtration of the dilute protein solution (150 ng/mL) at 1 mL/sec. The filtrate for each device was collected; 100 µL samples were pipetted into separate scintillation vials and counted. The data was normalized as described in Figure 1. Error bars indicate standard deviation (n = 10 Acrodisc syringe filters).*

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## DNA Transmission

To determine the transmission of DNA during filtration, radioactive-labeled PCR products were synthesized, purified, filtered and counted using liquid scintillation. Ultrafiltration was used to concentrate and desalt a 400 bp labeled product (Figure 3). This purification was necessary to remove unincorporated nucleotides so that they would not be counted as part of the transmitted activity. The purity of the $^{32}$P-labeled DNA is evident by the removal of the diffuse primer lower band sample after purification using a Nanosep® centrifugal device (PN OD100C33). The resulting DNA solution was used to test the transmission of DNA during normal filtration for Acrodisc filters containing Fluorodyne II, Supor, Ultipor, and Posidyne membranes.

**Figure 3**
Preparation of DNA Template for Transmission Studies

*A PCR labeling reaction was set up using 100 ng pUC18 template DNA, 20 nmole homologous primers, and PCR Supermix (Life Technologies, Gaithersburg, MD, USA) supplemented with 10 uCl $^{32}$P-dCTP. Synthesis resulted in a 400 bp low-specific activity product which was purified using a 100K Nanosep device to remove the unincorporated nucleotides (see Nanosep Centrifugal Devices: Protocols for Use, PN 32989; Nanosep Centrifugal Ultrafiltration Devices & PCR: Before & After). The unpurified PCR sample (A) contains primers and free-labeled nucleotides while the processed DNA sample (B) is free of unincorporated nucleotides.*

A total of 25 ng DNA in a 5 mL Tris EDTA solution was filtered through each device (Figure 4). The filtrate for each sample was collected and a 100 µL sample was counted using liquid scintillation (n = 30).

Fluorodyne II (PVDF), Supor (PES), and Ultipor (uncharged Nylon 6,6) all transmitted virtually 100% of the dilute DNA solutions. As expected, the Posidyne (positively-charged Nylon 6,6) membrane did bind the negatively-charged DNA and had the lowest transmission for the membranes tested (Figure 4).

**Figure 4**
Dilute DNA Transmission Through Select 25 mm Devices

*Each Acrodisc syringe filter was challenged with 5 mL (5 ng/mL DNA) during filtration at 1 mL/sec. The filtrate for each device was collected and 100 µL samples were pipetted into separate scintillation vials and counted in a multilabel counter. The data was normalized against unfiltered controls sampled at the beginning and end of each sampling. Error bars indicate standard deviation (n = 30 Acrodisc syringe filters).*

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## Conclusion

Acrodisc syringe filters containing Fluorodyne II, Supor, Ultipor, and Posidyne membranes are a central component of Pall's UpScale program. We have used filtration conditions consistent with user applications to show that the high performance, low-binding Fluorodyne II and Supor membranes allow biomolecule transmission to be maximized. When solutions containing trace amounts of biomolecules require chemical compatibility and high transmission, the Ultipor membrane can be passivated using a dilute nonionic detergent. In this study, the transmission characteristics of the Posidyne membrane were used as a positive control because it was known for its ability to bind biomolecules. Based on its ability to effectively bind biomolecules during filtration, the Posidyne membrane can be useful in enhancing bioburden and pyrogen removal from aqueous solutions.

The availability of small-volume R&D filters, made with the identical materials as the larger filters, allows reliable and rapid scale-up of the filtration steps required by the biopharm industry.

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