Efficient concentration of a low-titer bovine IgG with high recovery in low volume

This application note describes the design of circuits with low working volume, allowing for high concentration of dilute feed streams. The use of ReadyCircuit™ single-use assemblies allowed for aseptic connectivity of the filtration device. Filtration was performed using ReadyToProcess™ hollow fiber filter cartridges. The described process enabled concentration of a bovine IgG antibody from 50.0 to 0.165 L in less than 5 h.

Introduction

In production processes for clinical phase trials, many target molecules are expressed in low titers and might need to be concentrated before further processed. However, 200- to 500-times concentrations of dilute feed streams demand flexible system use and close control of the hold-up volume.

Here, we describe the use of ReadyCircuit disposable assemblies in the design of circuits with low working volumes to enable high concentration factors. The goal was a 500-times concentration of a target molecule in the shortest possible process time and within a closed system to minimize recirculation and hold-up volumes. A low-titer IgG antibody sample was used as staring material.

Filtration was performed on ReadyToProcess hollow fiber filter cartridges. Hollow fiber filters are especially suited for processes where the process stream needs to be contained for health and safety reasons. When using single-use assemblies, all process components that have been in contact with the process material, including the filter cartridge, can be conveniently disposed after use without the need for open handling of the product. Cassette filters, on the other hand, often require user-installation into a manifold assembly. The cassette and manifold assembly must be decontaminated during removal and disposal to prevent user exposure to potentially harmful process fluids. ReadyToProcess hollow fiber filter cartridges are available in a wide range of base unit configurations, allowing for easy selection and incorporation into a single-use assembly. The designed circuit can be used with any ReadyToProcess hollow fiber filter cartridge for concentration of dilute targets to high concentrations in low volume.

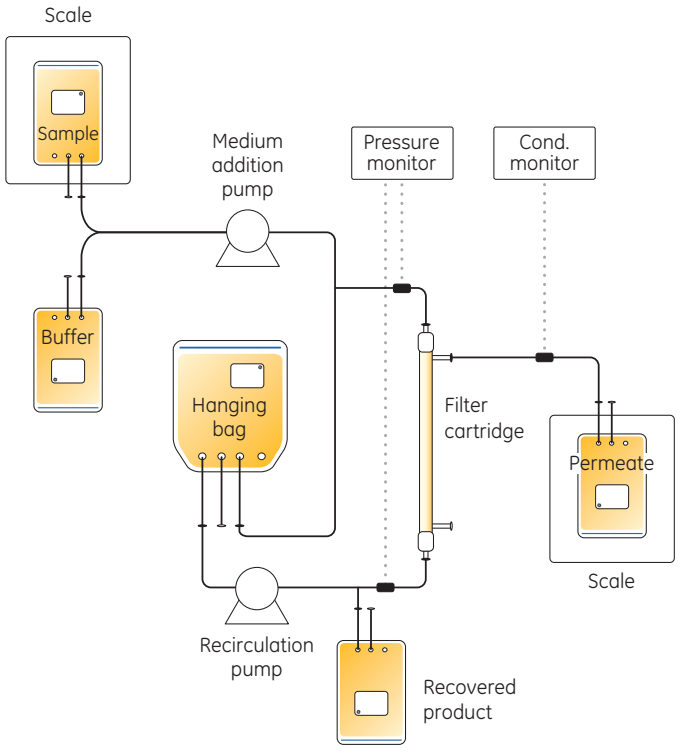

Fig 1. Schematic drawing of the filtration setup.

The described two-step filtration process enabled concentration of the antibody from 50.0 to 0.165 L with 100% recovery in less than five hours. An overview of the filtration setup is shown in Figure 1.

Materials and methods

Sample preparation

Commercially available bovine IgG was dissolved in phosphate buffered saline (PBS), pH 7.4. To remove any particles, the IgG-containing solution was filtered through a 0.2 µm filter before the concentration step.

Protein concentration was determined by measuring the absorbance at 280 nm and calculating the concentration using Beers law ($A = \varepsilon\ c\ l$), with $\varepsilon = 1.38$ mL mg^{-1} cm^{-1} (γ-globulin) and $l = 1$ cm.

Step 1 concentration and diafiltration

For the initial concentration from 50 to 1.3 L, followed by a diafiltration step to yield a final sample volume of 2 L, a ReadyToProcess hollow fiber filter cartridge of size 6 was used. The process was performed at a shear rate of 6000 s^{-1} and a transmembrane pressure (TMP) of 0.8 bar using stand-alone pumps (Watson-Marlow) and pressure sensors (Pendotech). The circuit was assembled as shown in Figure 2. Connection to the filter cartridge were secured in place with nylon Tri-Clamp™ fittings. Sample was held in a 100 L ReadyCircuit bag placed on a scale and connected to one branch of the ReadyMate™ three-way manifold on the medium addition line. The diafiltration buffer bag was connected to a second branch of the ReadyMate three-way manifold on the medium addition line and secured with a Tri-Clamp fitting. Tubing was connected with ReadyMate connectors from the permeate line of the filter to a 100 L waste container placed on a scale.

The protein solution was transferred to the recirculation bag using a calibrated pump. A flow rate of 325 mL/min for 4 min was used to accurately dispense 1.3 L of protein solution into the 5 L bag. With the permeate line and low-point drain lines clamped, the system was set to recirculate for 2 min at 2 L/min to remove air from the system. Slowly increasing the recirculation flow rate to the desired flow rate of 3.3 L/min (6000 s^{-1} shear rate), the circuit integrity was verified via visual inspection. With recirculation flow constant, the permeate line was unclamped and concentration of the proteins solution was started. While monitoring pressures, conductivity, permeate flow rate, and volumes, the system was slowly directed to the 12 psi (0.8 bar) target TMP by slowly increasing the retentate pressure using a c-clamp that evenly depresses the line for steady pressure control. The process was designed as a fed-batch operation, where the liquid line in the reservoir was maintained at a constant level below the exhaust air filter line on the reservoir bag until all 50 L of dilute IgG feedstock was dispensed into the system, upon which the medium addition pump was stopped and the concentration process was allowed to continue until the target sample volume of 1.3 L was achieved.

For diafiltration, the fluid addition line was switched from the feed bag to diafiltration buffer (0.1 M glycine + 0.1 M NaCl, pH 4.5), while maintaining the recirculation flow at 3.3 L/min and keeping TMP constant at 12 psi (0.8 bar). The fluid addition pump was started and the flow rate was

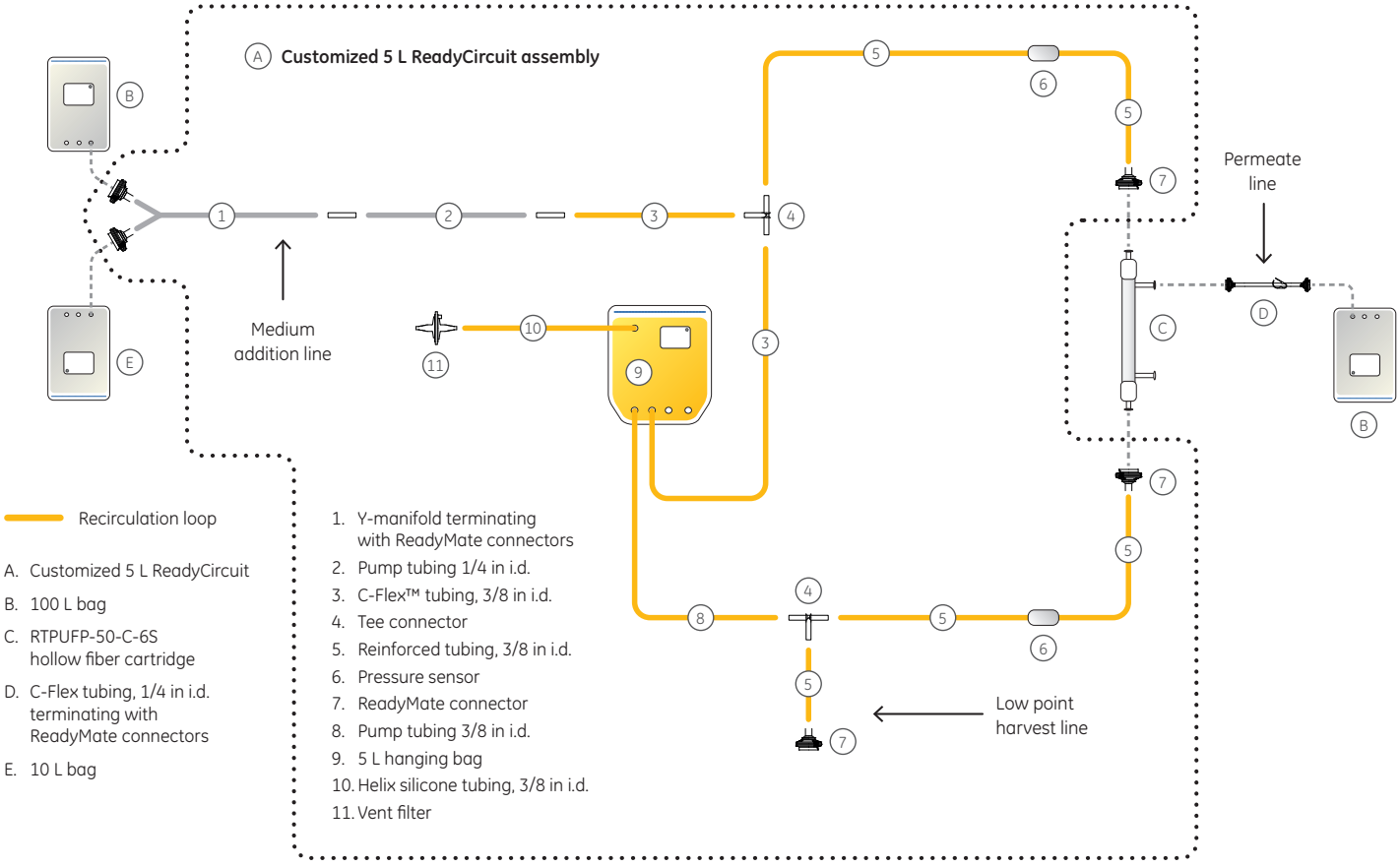

Fig 2. Diagram of the 50 L to 1.3 L circuit, with the customized 5 L ReadyCircuit assembly shown within dotted line. The total hold-up volume of the recirculation loop was 250 mL.

matched with the permeate flow rate to keep the sample volume constant at 1.3 L until 5.2 L diafiltration buffer was collected in the permeate waste. Conductivity determination of the reservoir content confirmed that the conductivity matched that of the diafiltration buffer, defining the endpoint of the diafiltration process.

When endpoint of diafiltration was reached, the medium addition pump was stopped and the retentate pressure was released by removing the clamp from the retentate line. The permeate line was clamped to stop flow out of the system and the system was recirculated for approx. 2 min before the recirculation flow rate was slowly decreased to 1 L/min. Thereafter, the low-point drain was connected to a sample collection container and the flow was directed to empty the recirculation loop. For a final flush, the diafiltration line was isolated and the medium pump was started and set to 350 mL/min for 2 min for addition of 700 mL for a final sample volume of 2 L. Recirculation was again set to a flow rate of 3.3 L/min for approx. 2 min to recover remaining material from the circuit. Thereafter, the recirculation line was removed from the recirculation pump and air was forced into the vent line with the use of a 60 mL syringe to allow for flow of material to the collection container.

Step 2 concentration

For sample concentration from 2 L to 0.1 L, a ReadyToProcess hollow fiber cartridge size 4M was used. The process was performed at shear rate of 6000 s^{-1} and a TMP of 0.3 bar using stand-alone pumps (Watson-Marlow) and pressure sensors (Pendotech). The circuit was assembled as shown in Figure 3. Connections to the inlet and retentate of the filter cartridge were secured in place with nylon Tri-Clamp fittings. Process fluid was held in a 5 L ReadyToProcess bag placed on a scale and connected to one branch of the three-way ReadyMate connector on the medium addition line and secured with a Tri-Clamp fitting. The diafiltration buffer bag (for recovery flush) was connected to a second branch of the three-way ReadyMate connector on the medium addition line and secured with a Tri-Clamp fitting. Tubing was connected with ReadyMate connectors from the permeate line of the filter to a 5 L waste container placed on a scale.

The fluid addition pump was set to deliver protein solution until volume in reservoir was just below the air vent line of the bag. With the permeate line and low point drain lines clamped, the system was set to recirculate for 2 min at 0.5 L/min to remove air from the system. Slowly increasing the recirculation flow rate to the desired flow rate of 0.9 L/min (6000 s^{-1} shear

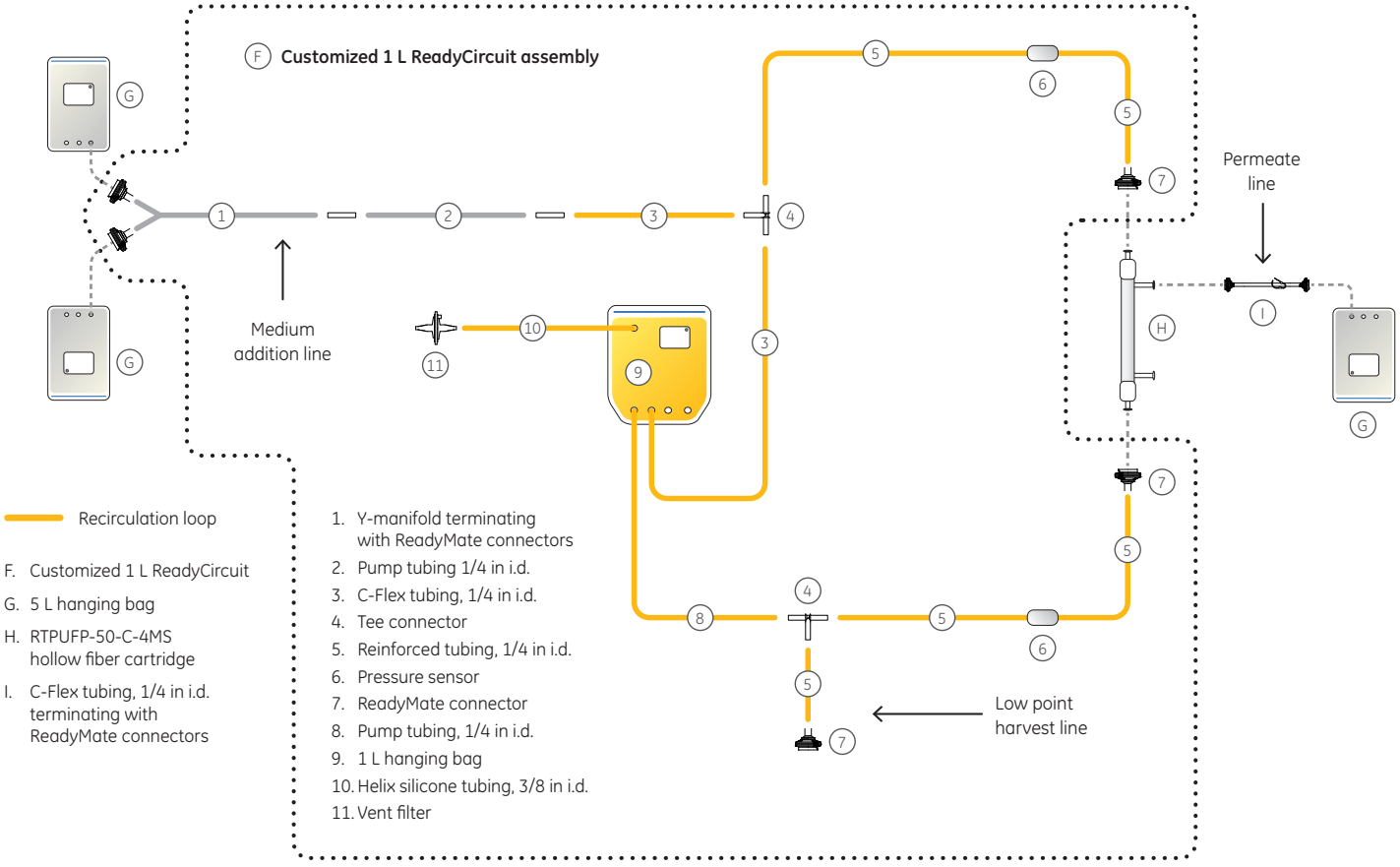

Fig 3. Diagram of the 2 L to 0.1 L circuit, with the customized 1 L ReadyCircuit assembly shown within dotted line. The total hold-up volume of the recirculation loop was 70 mL.

rate), the integrity was verified via visual inspection. With recirculation flow constant, the permeate line was unclamped and concentration of the proteins solution was started. While monitoring pressures, conductivity, permeate flow rate, and volumes, the system was slowly directed to the 4 psi (0.3 bar) target TMP by slowly increasing the retentate pressure using a c-clamp. The process was designed as a fed-batch operation, where liquid line in the reservoir was maintained at a constant level below the exhaust air filter line on the reservoir bag until all 2 L sample was dispensed into the system, upon which the medium addition pump was stopped and the concentration process was allowed to continue until the target volume (0.1 L) was achieved.

Before endpoint of concentration was reached, the retentate pressure was released by removing the clamp from the retentate line to avoid over-concentration of the sample. When endpoint of concentration was reached, permeate line was clamped to stop flow out of the system and the system was recirculated for approx. 2 min before the recirculation flow rate was slowly decreased to 0.3 L/min. Thereafter, the low-point drain was connected to a sample collection container and the flow was directed to empty the recirculation loop. For a final flush, the recirculation line was isolated and the fluid addition pump was started and set to 35 mL/min for 2 min for addition of 70 mL for a final sample volume of 165 mL. Recirculation was again set to a flow rate of 0.9 L/min for approx. 2 min to recover remaining concentrated IgG from the circuit. Thereafter, the recirculation line was removed from the recirculation pump and air was forced into the vent line with the use of a 60 mL syringe to allow for flow of material to the collection container.

Results

A schematic overview of the two-step process for concentration and diafiltration of a bovine IgG antibody is shown in Figure 4.

Figure 5 shows TMP, flux, and concentration factor for the first step sample concentration from 50 to 1.3 L and diafiltration to a final sample volume of 2 L. As shown, flux rate slowly decreased as the concentration of the product increased. TMP was maintained at approx. 12 psi (0.8 bar) for the entire run. The average flux for the run was determined to be 46 L/m^2/h and the total process time was about 2.3 h.

The recovery of this process step was about 100%. One single flush was sufficient to achieve this high recovery. The concentration factor for the process was 44×. After a final flush, 2 L of partially concentrated IgG solution was recovered.

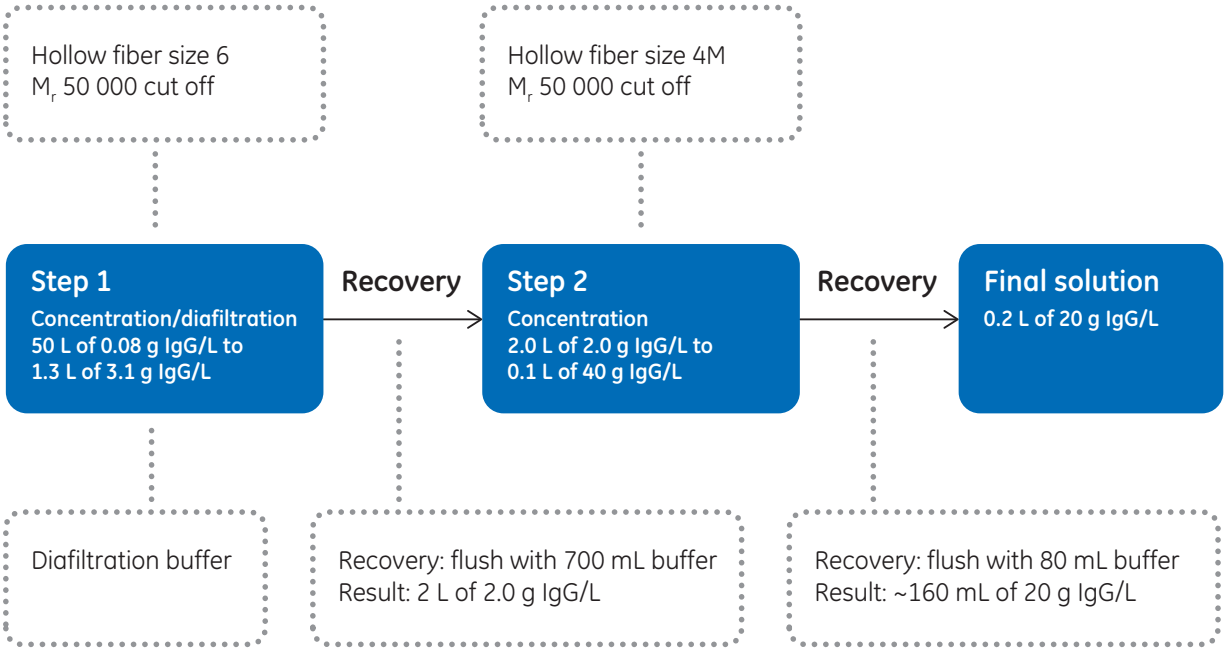

Fig 4. Schematic overview of the two-step concentration and diafiltration of bovine IgG.

Figure 6 shows TMP, flux, and concentration factor for the second step sample concentration from 2 to 0.1 L. TMP was maintained at approx. 4 psi (0.3 bar) for the entire run. When concentration factor reached 225×, the flux dropped about 30%, from 17 to 12 L/m²/h but levelled off over the rest of the run. Average flux for this process step was 16 L/m²/h and the total process time was about 1.9 h.

The recovery of this second process step was also about 100%. One single flush was sufficient to achieve this high recovery. Concentration factor for the process was 20×. After recovery flush, the final volume of the concentrated IgG solution was 165 mL.

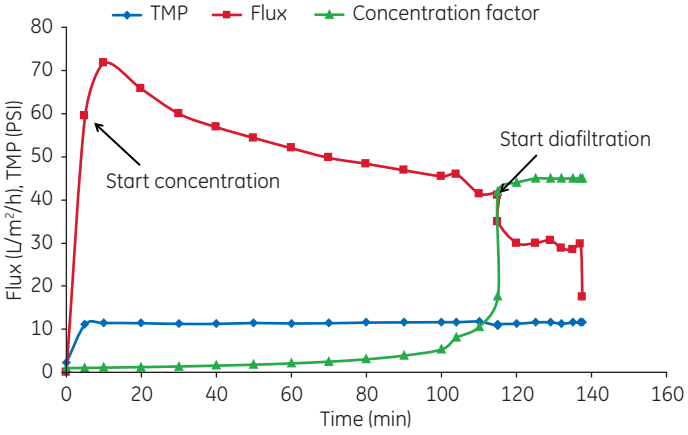

Fig 5. Step 1 sample concentration from 50 to 1.3 L, followed by diafiltration and recovery flush to a final sample volume of 2 L.

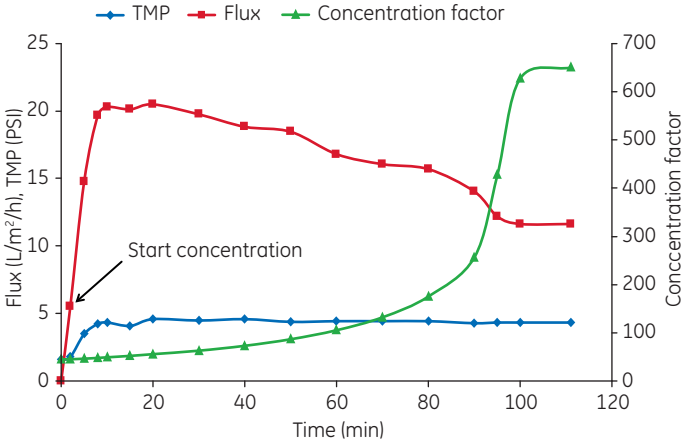

Fig 6. Step 2 sample concentration from 2 to 0.1 L.

Summary of process results

In the described two-step filtration process, a bovine IgG antibody was concentrated 650 times with a total recovery of 100% (Table 2). The process time for the first and second steps was 2.3 h and 1.9 h, respectively; giving a total process time of 4.2 h. Time for assembly of each circuit was approx. 30 min, including setup and connectivity. Disassembly and disposal time for the circuits was determined to be approximately 10 min each. Compared with reusable equipment, cleaning is eliminated as the single-use circuits can be completely disposed after use.

Table 2. Summary of results from the two-step IgG concentration process

Sample	IgG conc. (mg/mL)	Volume (mL)	Recovery (%)*
Starting sample	0.1	50000	100
Step 1: retentate	3.2	1200	104
Step 1: flush	0.4	700	7
Step 1: retentate + flush	2.0	1890	105
Step 2: retentate	40.6	80	86
Step 2: flush	8.0	90	19
Step 2: retentate + flush	23.1	165	105

*Concentration > 100% likely due to measurement accuracy limitations.

Conclusion

Here, we describe a two-step filtration process for concentration of a low-titer IgG to high concentration in low volume. The filtration circuits were specially designed with lowest possible working volumes to achieve a high concentration factor. The described process allowed a final concentration factor of more than 500 times, with a sample recovery of 100%, in less than 5 h. Important for such a high recovery is the low point drain. For full sample recovery, air was forced into the system to allow complete emptying of the circuit. The use of ReadyCircuit assemblies enabled aseptic connectivity of the filtration device. The successful use of stand-alone pumps and pressure sensors for process operations demonstrates flexibility in system use.

The described process can be used as starting point, keeping in mind that when concentrating larger targets such as viral vaccines or virus-like particles, membranes of larger pore sizes might be required, resulting in a higher flux and a shorter process time. The described process, yielding a final volume of 0.165 L from a starting volume of 50.0 L, will allow users to concentrate their target, whether it is a viral vaccine or protein product, for final fill activities and clinical trial applications.

Ordering information

Product	Identifier	Required quantity	Product code
Step 1			
Customized 5 L ReadyCircuit	A	1	RC2016-0132
100 L ReadyCircuit bag	B	2	29064848
RTPUFP-50-C-6S	C	1	39000036
3 ft jumper assembly	D	1	12410115
10 L ReadyCircuit bag	E	1	12410222
Step 2			
Customized 1 L ReadyCircuit	F	1	RC2016-0134
5 L ReadyToProcess bag	G	3	12410220
RTPUFP-50-C-4MS	H	1	39000069
3 ft jumper assembly	I	1	12410115

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