NCSRT

Works™ Protocol

Isolation of secreted monoclonal antibodies from mammalian cells

This *Isolation of secreted monoclonal antibodies from mammalian cells* protocol is intended for isolating, concentrating and diafiltering recombinant proteins such as monoclonal antibodies (Mab) in two simultaneous processes. This process has been repeatedly implemented with consistent success in CHO cell culture.

The initial step isolates the target Mab from the culture medium by using a 0.45 µm modified polyethersulfone membrane (MPS) to pass the Mab freely into the permeate and retain the cells, large molecular weight broth components, and any accumulated cell debris. The protocol calls for the media to be concentrated to 5X prior to starting the diafiltration. The required diafiltration buffer is generated in the second (ultrafiltration) process.

The target of the second step is to concentrate the target molecule with a 50kD polyethersulfone (PES) ultrafiltration membrane. The permeate from this process is fed back to the recirculation loop of the isolation process to create a closed loop system. The target product is concentrated in the retentate tank of the second loop and recovered when the desired concentration or target yield is achieved.

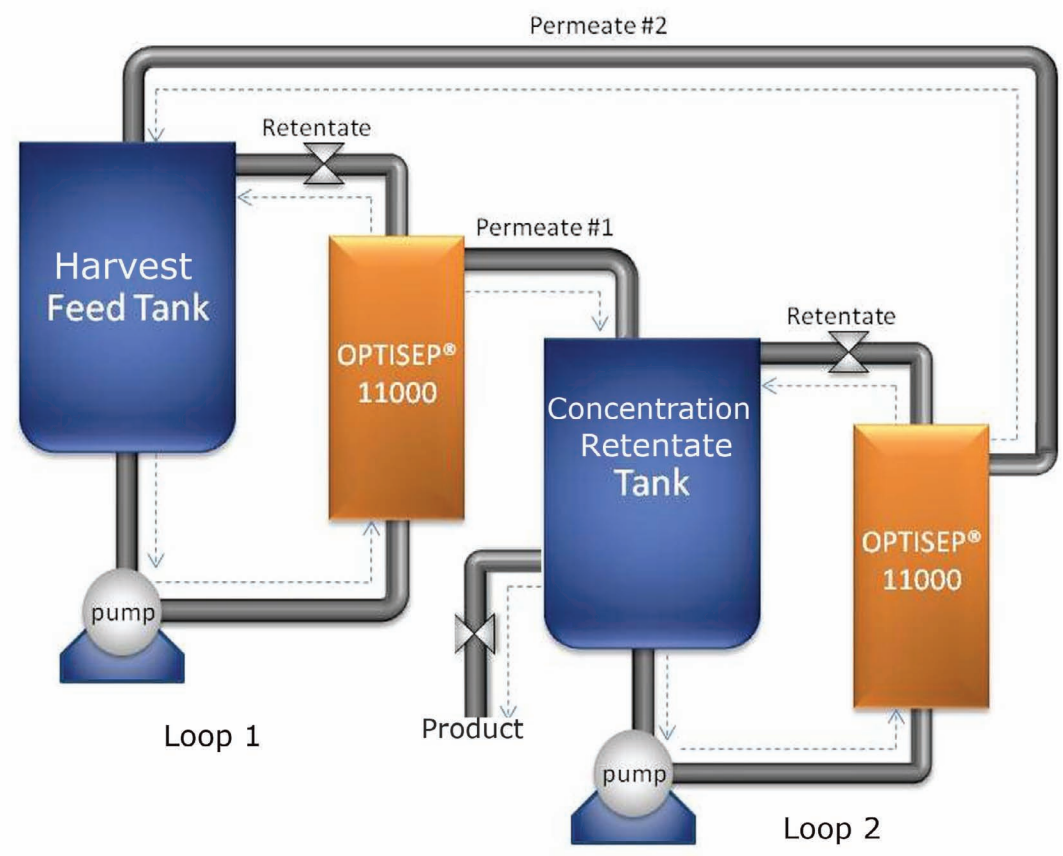

Figure 1 – Simultaneous Processing Schematic

OPTISEP® filtration module with MPS 0.45 µm membrane. Solids are retained, product in permeate.

OPTISEP filtration with PES 50kD UF membrane. Product is retained, low molecular weight components are in the permeate.

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Process Conditions:

Product: Mab
Process Objective: Isolation from cell culture media with a batch size ranging from 100-1000L.
Procedure: Concentrate the starting material 5X and perform a 5X diafiltration
Isolation Loop Filter: OPTISEP® 11000 0.45 µm membrane, 0.75 mm channel height
Isolation Loop Velocity: 100 cm/sec
Concentration Loop Filter: OPTISEP 11000 PES 50 kD UF membrane, 0.75 mm channel height
Concentration Loop Shear: 10,000 sec^{-1}
Expected Yield: >95% product yield

Enter the fermentation broth volume to be used in the isolation loop fill in the following table:

Table 1 Membrane area determination – isolation loop

	A	B	C	D	E		
	Starting Volume (liters)	LM* for isolation step	Membrane area required (Col A/ Col B)	OPTISEP 11000 filter module (9.8m^2) 0.45 µm MPS 0.75 channel height	Velocity of retentate at the membrane surface (cm/sec)	Shear sec^{-1}	Recirculation flow rate (per 9.8 m^2 OPTISEP 11000 module)
Production		60		74-E 1N-9045	100	6,470	260 l/min (70 gpm)

* L starting material/ m^2 membrane area

The isolation loop uses the OPTISEP 11000 module with MPS 0.45 µm MF membrane and 0.75 mm channel height to concentrate the process stream 5X. The process volume for the first step is determined by the fermentation volume. The required membrane area is determined by dividing the starting volume by 60 LM (Table 1).

Example: 500 L fermentation / 60 LM = 8.3 m^2
Purchase 1 100 ft^2 (9.8 m^2) OPTISEP 11000 filter module.

Run the process at 260 l/min per 100 ft^2 (9.8m^2) module. Begin the process by slowly bringing the recirculation pump up to the calculated recirculation rate. The outlet pressure should be zero (0). The inlet pressure should be around 10 psi or less. Collect the permeate of the isolation loop in the retentate reservoir of the concentration loop.

When the isolation loop reaches 2X concentration, start the concentration loop to capture the target product.

The concentration loop utilizes OPTISEP 11000 modules with PES 50 UF membrane and a 0.75 mm channel height. The concentration loop contains twice the membrane area of the isolation loop because the small pore size membrane tends to have a slower flux than the larger pore size membrane used for the product isolation. The flux in the concentration loop must be able to exceed the permeate flow rate in isolation step to maintain an uninterrupted source of diafiltration buffer. Adjust the pump speed to 400 L/min (105 gpm) per 100 ft^2 (9.8m^2) OPTISEP 11000 module. Adjust the backpressure to achieve 20 psi back pressure. If the permeate flow is higher than the diafiltration needs of the isolation step, the pressure can be lowered. As the system pressure is

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Table 2: Concentration loop calculations

	A	B	C	D	E		
	Starting Volume (liters)	LM* for isolation step	Membrane area required (Col A/ Col B)	OPTISEP® 11000 filter module (9.8 m^2) PES 50 kD 0.75 mm channe height	Velocity of retentate at the membrane surface (cm/sec)	Shear sec^{-1}	Recirculation flow rate (per 9.8 m^2 OPTISEP 11000 module)
Production		60		74-E 2B-0050	155	10,000	400 l/min (105 gpm)

* L starting material/ m^2 membrane area

changed, the flow rate of the pump may change. Be sure to adjust the pump speed to maintain the required recirculation rate.Based on the permeate flow in the isolation step, run both systems simultaneously for the required period of time to perform a 5X diafiltration the product in the isolation loop.

After the 5 X diafiltration is complete, the isolation loop may be cleaned. Remember to remove the permeate line from the concentration loop retentate tank. The permeate line from the concentration loop may be moved from the retentate tank of the concentration loop and directed to waste.

Concentrate the product in the concentration loop to the desired level. To increase the process yield, over concentrate the product in the concentration loop by one system volume and use a one volume of system flush to recover product residue remaining in the system after draining. To maximize the effectiveness of the rinse, recirculate the rinse buffer at half the process recirculation rate (200 L/min per OPTISEP 11000 module) for 5 minutes with the backpressure set to zero and the permeate line going back to the feed tank.

For small scale verification of the *Isolation of secreted proteins from mammalian cells* protocol prior to scale up Table 3 contains the products and process conditions to perform a 60L trial using 10 ft^2 (.98 m^2) OPTISEP 11000 modules.

Execute the process steps above at the 60L starting volume. This will require a minimum retentate tank for the concentration loop of 30L.

Table 3 – Small scale protocol evaluation requirements

	Starting Volume (liters)	LM	Membrane area required (Col A/ Col B)	OPTISEP 11000 filter module (10 ft^2 (0.9 m^2))	Velocity of retentate at the membrane surface (cm/sec)	Shear sec^{-1}	Recirculation flow rate	TMP
Isolation Loop	60	60	1.0	71-E1N -9045 MPS 0.45 µ 0.75 gasket	100	6,470	30 L/min (8 gpm)	< 6 PSI
Concentration Loop	30	15 Determined by isolation	2.0	71-E2B - 0050 PES 50 kD 0.75 gasket Two (2) required	155	10,000	95 L/min (25 gpm)	Set to achieve needed permeate flow rate.

If the results from the small scale verification runs are unacceptable or there is the desire to optimize the process for the target molecule, perform the systematic evaluation of alternative membranes and process con-

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dition described in the *Isolation of secreted proteins from mammalian cells* Optimization Procedure from NCSRT.

To learn how others have applied the patented *SmartFlow*™ filter modules technology to their separations, consult the *Isolation of secreted proteins from mammalian cells* case study.

Product Ordering Information

Description	Part Number
OPTISEP® 11000 holder	70-900-2300
OPTISEP 11000 filter module MPS 0.45µm membrane 0.75 mm channel 100 ft^2 (9.8 m^2)	74-E1N-9045
OPTISEP 11000 filter module MPS 0.45µm 0.75 mm channel 50 ft^2 (4.9 m^2)	72- E1N-9045
OPTISEP 11000 filter module MPS 0.45µm 0.75 mm channel 10 ft^2 (0.9 m^2)	71- E1N-9045
OPTISEP 11000 filter module PES 50 membrane 0.75 mm channel 100 ft^2 (9.8 m^2)	74-E2B-0050
OPTISEP 11000 filter module PES 50 membrane 0.75 mm channel 50 ft^2 (4.9 m^2)	72- E2B-0050
OPTISEP 11000 filter module PES 50 membrane 0.75 mm channel 10 ft^2 (0.9 m^2)	71- E2B-0050
Cart for OPTISEP 11000 holder	0050-53-02

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