NCSRT

Works™ Protocol

Isolation of proteins (<30Kd) from cell lysate with *SmartFlow*™ TFF

This *Isolation of proteins from cell lysate* protocol is intended for isolating, concentrating and diafiltering recombinant proteins with a molecular weight of less than 30 kD from lysed cells in two simultaneous processes. This process has been repeatedly implemented with consistent success in *E.coli* and *Pichia* fermentations.

The initial step isolates the target molecule from the fermentation broth by using a 100 kD regenerated cellulose membrane to pass the target molecule freely into the permeate and retain the large molecular weight broth components, and the accumulated cell debris. The protocol call for the fermentation broth 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 5kD regenerated cellulose 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.

Process Conditions:
Product: Protein (< 30 kD) from fermentation
Process Objective: Isolation from cell lysate with a batch size ranging from 100-1000L.
Procedure: Concentrate the starting material 5X and perform a 5X diafiltration
Isolation Loop Filter: OPTISEP® 11000 RC 100 kD UF membrane, 0.75 mm channel height
Isolation Loop Shear: 10,000 sec $^{-1}$
Concentration Loop Filter: OPTISEP 11000 RC 5 kD UF membrane, 0.5 mm channel height
Concentration Loop Shear: 10,000 sec $^{-1}$
Expected Yield: >95% product yield

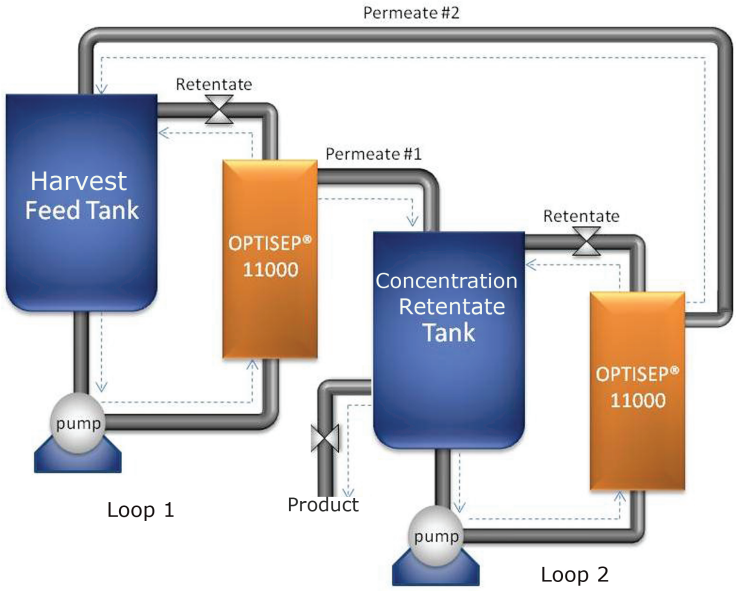

Figure 1 – Simultaneous Processing Schematic

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	RC 100 Membrane area required (Col A/ Col B)	OPTISEP 11000 filter module (9.8 m²) RC 100 kD 0.75 gasket	Velocity of retentate at the membrane surface	Shear sec $^{-1}$	Recirculation flow rate (per 9.8 m² OPTISEP 11000 module)
Production		60		74-E5B-0100	1.0	10,000	380 L/min (100 gpm)

* Liters per m² membrane area

Isolation of proteins from cell lysate

The isolation loop uses the OPTISEP® 11000 module with RC 100 UF 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 m²
Purchase 1 100 ft² (9.8 m²) OPTISEP 11000 filter module.

Run the process at 380 L/min per 100 ft² (9.8m²) module with 10 psig (0.69 bar) inlet pressure and 0 psig (0 bar) outlet pressure. This will result in a TMP of 5 psig (0.34 bar)
Collect the permeate from the isolation loop in the recirculation reservoir for 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 RC 5 UF membrane and a 0.5 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 136 L/min (36 gpm) per 100 ft² (9.8m²) OPTISEP 11000 module. Adjust the TMP to achieve a back pressure of 60 psi. If the permeate flow is higher than the diafiltration needs of the isolation step, the pressure can be lowered. As the system pressure is changed, the flow rate of the pump may change. Be sure to adjust the pump speed to maintain the required recirculation rate.

Table 2 - Concentration loop calculations

	A	B	C	D	E			
	Starting Volume (liters)	LM for concentration step	RC 5 Membrane area required Col A / Col B	OPTISEP 11000 filter module (9.8 m²)	Velocity of retentate at the membrane surface	Shear sec^{-1}	Recirculation flow rate (per 9.8 m^2 OPTISEP 11000 module)	TMP
Production			Twice the membrane area of Loop 1	74-D5B-0005	1.0 m/sec	10,000	136 L/min (36 gpm)	Set to achieve needed permeate flow

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 5X 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 (68 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 proteins from cell lysate* protocol prior to scale up, Table 3 contains the products and process conditions to perform a 60L trial using 10 ft² (.98 m²) 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 for isolation step	RC 100 Membrane area required (Col A/Col B)	OPTISEP 11000 filter module (10 ft² (0.9 m²)) RC 100 kD	Velocity of retentate at the membrane surface	Shear sec^{-1}	Recirculation flow rate	TMP
Isolation Loop	60	60	1.0	71-E5B-0100 0.75 gasket	1.0	10,000	35.6 L/min (9.4 gpm)	5
Concentration Loop	30	15 Set by isolation step.	2.0	71-D5B-0005 Two (2) required 0.5 gasket	1.0	10,000	25.5 L/min (6.72 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 condition described in the *Isolation of proteins from cell lysate WORKS*™ optimization procedure from NCSRT. To learn how others have applied the patented *SmartFlow*™ filter modules technology to their separations, consult the *Isolation of proteins from cell lysate WORKS* case study.

Description	Part Number		
		OPTISEP 11000 filter module RC100 membrane 0.75 mm channel	OPTISEP 11000 filter module RC 5 membrane 0.5 mm channel
OPTISEP 11000 filter 100 ft² (9.8 m²)		74-E5B-0100	74-D5B-0005
OPTISEP 11000 filter 50 ft² (4.9 m²)		72-E5B-0100	72-D5B-0005
OPTISEP 11000 filter 10 ft² (1.0 m²)		71-E5B-0100	71-D5B-0005
OPTISEP 11000 holder	70-900-2300		
Cart for OPTISEP 11000 holder	0050-53-02		

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