NCSRT

Works™ Protocol

Secreted proteins from whole cells with *SmartFlow*™ TFF

This *Secreted proteins from whole cell* protocol is intended for isolating a product secreted product from either a bacteria or yeast. This process has been repeatedly implemented with consistent success in whole cell microbial fermentations with products less than 80 K molecular weight.

The protocol describes the isolation of the target molecule from the fermentation broth using a high molecular weight ultrafiltration membrane to pass the target molecule freely into the permeate and retain the cells, large molecular weight broth components, and any accumulated cell debris. The protocol calls for the fermentation broth to be concentrated 2X or to an OD of 400 prior to starting the diafiltration.

Secreted proteins from whole cell protocol:
Product: 3-80k MW Product
Process Objective: Isolation from fermentation broth ranging from 100-1000L.
Procedure: Concentrate the starting material 2X and perform a 3X diafiltration.
Membrane: RC 100 for products < 30 kD. MPS500 for products >30kD and < 80 kD.
Expected Yield: >95% product yield.

Enter the fermentation broth volume to be used in column A of the following table and calculate the membrane area in column C.

Table 1 - Membrane area determination

A	B	C	D	E		
Starting Volume (liters)	LM *	Membrane area required (Col A/ Col B)	OPTISEP® 11000 filter module (9.8 m²) 0.75 mm gasket	Velocity of retentate at the membrane surface	Shear sec^{-1}	Recirculation flow rate (per 9.8 m² OPTISEP 11000 module)
	60		RC100 74-E5B-0100 MPS 500 74-E1N-B100	100 cm/sec	6,470	260 l/min (70 gpm)

* L starting material/ m² membrane area

An OPTISEP module with 0.75 mm channel height is used to concentrate the process stream 2X. The required membrane area is determined by dividing the starting volume by 60 LM (Table 1). Round this number up to the nearest liter if it is below 5 and up to the nearest 5 if it is above 5. For example, to run a 100 l batch, you need 100/60 = 1.7 m², which rounds to 2 m². For a 1000 l batch, you need 1000/60 = 16.7 m², which rounds up to 20 m². Refer to Table 3 for the appropriate part numbers for ordering.
Example: 500 L fermentation / 60 LM = 8.3 m²
Purchase 1 100 ft² (9.8 m²) OPTISEP 11000 filter module.

Run the process at 260 l/min (70 gpm). Slowly increasing the recirculation rate until the desired recirculation rate is reached. The inlet pressure should be between 6 psig (0.4 bar) and 12 psig (0.8 bar). If the inlet pressure is less than 6 psig (0.4 bar), apply back pressure by closing the back pressure valve until an inlet pressure of 6 psig (0.4 bar) is reached.

Collect the permeate in an appropriate vessel for the next process step. When the starting material reaches 2X concentration, start the diafiltration. Add the diafiltration buffer at the same rate as the permeate is leaving the system. To determine the volume of diafiltration buffer, calculate the starting volume divided by the concentration factor and multiply that number by 3. After the 3 X diafiltration is complete, the diafiltration may be stopped and the system may be cleaned.

The target product is contained in the permeate. If desired, concentrate the product in the permeate reservoir to the desired level using the NCSRT protocol for *Ultrafiltration,concentration, and diafiltration*. Alternatively, if the product isolation step is to be immediately followed by a UF concentration of the product, see NCSRT's *Simultaneous concentration and diafiltration* protocol for a method to greatly reduce the volume of the diafiltration buffer that needs to be prepared and the size of the tanks needed for the diafiltration and product collection.

Small Scale Trial:
For small scale verification of the *Secreted proteins from whole cell* protocol prior to scale up, Table 2 contains the products and process conditions to perform a 60L trial using 10 ft² (0.9 m²) OPTISEP® 11000 modules. Execute the process steps above at the 60L starting volume. This will require a minimum permeate reservoir of 150L to perform the 2X concentration and a 3 X diafiltration.

2X concentration generates 30 L permeate.
3X diafiltration generates 90 L of permeate.
Total permeate is 120L.

Table 2

	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)) MPS 500 kD 0.75 gasket	Velocity of retentate at the membrane surface	Shear sec^{-1}	Recirculation flow rate	TMP
Product Isolation	60	60	1.0	71-E1N-B100	100 m/sec	6,470	30.7 l/min (8.1 gpm)	< 10

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 *Secreted proteins from whole cell* Optimization Procedure from NCSRT.

Table 3

Description	Part Number	Regenerated Cellulose 100K	Modified Polysulfone 500K
OPTISEP 11000 holder	70-900-2300		
OPTISEP 11000 filter module 0.75 mm channel100 ft2 (9.8 m2)		74-E5B -0100	74-E1N-B100
OPTISEP 11000 filter module 0.75 mm channel50 ft2 (4.9 m2)		72-E5B -0100	72-E1N-B100
OPTISEP 11000 filter module 0.75 mm channel 10 ft2 (0.9 m2)		71-E5B -0100	71-E1N-B100
Cart for OPTISEP 11000 holder	0050-53-02		