NCSRT

Works™ Protocol

Purification of secreted viral antigens from cell culture with *SmartFlow*™ TFF

This *Purification of secreted viral antigens from cell culture* protocol is intended for isolating a secreted whole virus or a viral antigen from a mammalian cell. This process has been repeatedly implemented with consistent success for isolating viral antigens excluding the smallpox, rodavirus, parvo, and hepatitis viruses.

The protocol describes the isolation of a whole virus or a secreted viral antigen from a cell culture media using a submicron microporous membrane to pass the target molecule into the permeate and retain the cells, large molecular weight broth components, and any accumulated cell debris. The protocol calls for the culture media to be concentrated 5X prior to starting the diafiltration.

Purification of secreted viral antigens from cell culture protocol:

Product: Secreted viral antigen or whole virus
Process Objective: Isolation from Mammalian cell culture broth ranging from 100-1000L.
Procedure: Concentrate the starting material 5X and perform a 3X diafiltration
Expected Yield: >95% product yield

Enter the cell culture media 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 ($9.8\ m^2$) 0.75 mm gasket	Velocity of retentate at the membrane surface	Shear sec^{-1}	Recirculation flow rate (per $9.8\ m^2$ OPTISEP 11000 module)
	60		0.45 µm MPS 74-E1N-9045	100 cm/sec	6,470	260 l/min (70 gpm)

* L starting material/ m^2 membrane area

An OPTISEP module with 0.75 mm channel height is used to concentrate the mammalian cell broth by 5X. 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 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 m^2, which rounds to 2 m^2. For a 1000 l batch, you need 1000/60 = 16.7 m2, which rounds up to 20 m^2. Refer to Table 3 for the appropriate part numbers for ordering.
Example: 500 L fermentation / 60 LM = 8.3 m^2
Purchase 1 100 ft^2 (9.8 m^2) OPTISEP 11000 filter module.

Begin the process by slowly increasing the recirculation rate until the desired recirculation rate is reached. Calculate the desired recirculation rate in LPM for OPTISEP 11000 filter modules. Run the process at 260 l/min per 100 ft^2 (9.8m^2) module). 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.

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Collect the permeate in an appropriate vessel for the next process step. When the starting material reaches 5X 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 WORKS™ 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* WORKS 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 *Purification of secreted viral antigens from cell culture* 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 100L to perform the 5X concentration and a 3 X diafiltration.

5X concentration generates 48 L permeate.
3X diafiltration generates 36L of permeate.
Total permeate is 84L.

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)) 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 - 9045	100 cm/sec	6,470	30.7 l/min (8.1 gpm)	< 5

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 *Purification of secreted viral antigens from cell culture* Optimization Procedure from NCSRT.

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To order *SmartFlow*™ filter modules and holders, please use Table 3 to assist in develping the correct part number.

Table 3

Description	Part Number	Modified Polysulfone 0.45 µm
OPTISEP 11000 holder	70-900-2300	
OPTISEP 11000 filter module 0.75 mm channel100 ft² (9.8 m²)		74-E1N-9045
OPTISEP 11000 filter module 0.75 mm channel50 ft² (4.9 m²)		72-E1N-9045
OPTISEP 11000 filter module 0.75 mm channel 10 ft² (0.9 m²)		71-E1N-9045
Cart for OPTISEP 11000 holder	0050-53-02	

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