NCSRT

Works™ Protocol

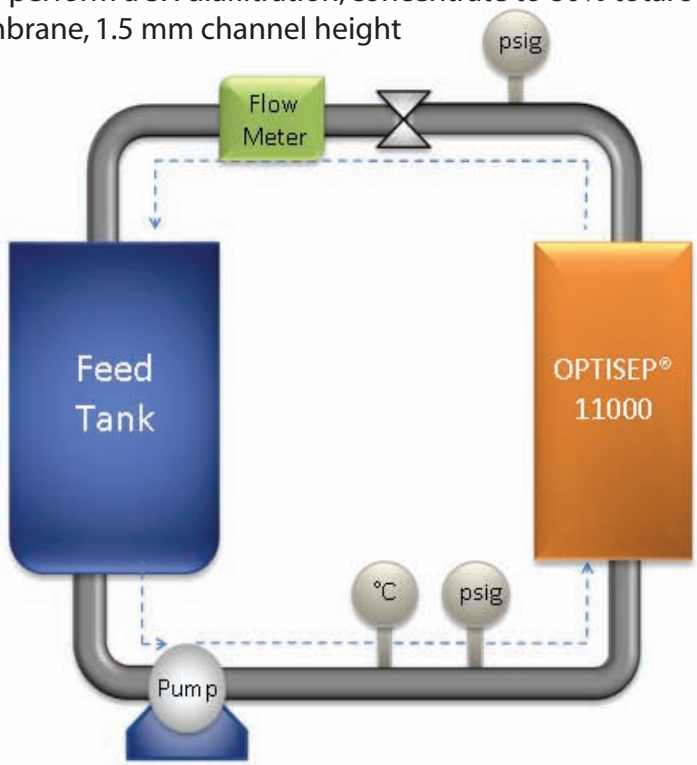
Concentration and diafiltration of whole cells

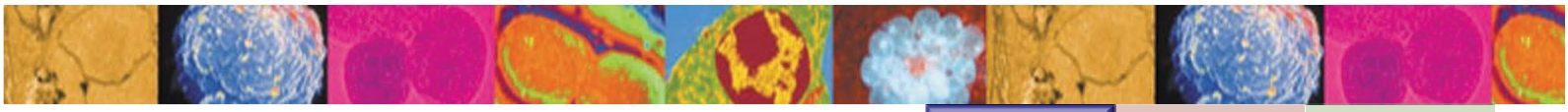
This *Concentration and diafiltration of whole cells* protocol is intended for isolating, concentrating and diafiltering recombinant cells from fermentation broth. This process has been repeatedly implemented with consistent success in *E. coli* and *Pichia* fermentation operations.

The filtration isolates the bacteria from the broth components with an ultrafiltration membrane (UF) to pass the broth components freely into the permeate and retain the cells. The protocol calls for the cells starting with an OD of 100 or less to be concentrated to 5X prior to starting the diafiltration. After diafiltration the cells are concentrated to a 80% total solids cell paste.

Process Conditions:

Product: Cell paste
Process Objective: Isolation of cells from fermentation broth in batch sizes ranging from 100-1000L.
Procedure: Concentrate the starting material 5X and perform a 3X diafiltration, concentrate to 80% total solids.
Isolation Loop Filter: OPTISEP 11000 RC100 kD membrane, 1.5 mm channel height
Isolation Loop Shear: 3,000 sec^{-1}
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) 1.5 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-J5B -0100	100 cm/sec	3,000	556 l/min (147 gpm)

* L starting material/ m² membrane area

The system uses the OPTISEP 11000 module with RC100 kD UF membrane and 1.5 mm channel height to concentrate the process stream and then perform a diafiltration. 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 556 L/min per 100 ft² (9.8m²) module. Begin the process by slowly bringing the recirculation pump up to the calculated recirculation rate. The outlet pressure should be set to 12 psi (0.83 bar). The inlet pressure should be around 20 psi (1.37 bar). The inlet pressure will increase during the experiment as the cells become thicker. The permeate can be discarded after taking the sample for analysis.

After the diafiltration is complete, the cells should be concentrated to 80% total solids. As the cells become more concentrated, the flux rate typically drops. Also the pressure drop down the channel will increase. By the end of the experiment a pressure drop up to 30 psi (2 bar) may occur across the filter.

For small scale verification of the *Concentration and diafiltration of whole bacterial cells* protocol prior to scale up Table 2 contains the products and process conditions to perform a 60L trial using 10 ft² (.98 m²) OPTISEP 11000 modules.

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)) RC 100 kD 1.5 gasket	Velocity of retentate at the membrane surface	Shear sec^{-1}	Recirculation flow rate	TMP
Isolation Loop	60	60	1.0	71-J5B -0100	100 cm/sec	3,000	65 l/min (17.3 gpm)	16

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 *Concentration and diafiltration of whole cells* Optimization Procedure from NCSRT.

To learn how others have applied the patented *SmartFlow*™ filter modules technology to their separations, consult the *Concentration and diafiltration of whole cells* Case Study.

To execute the described protocol at virtually any scale, please refer to Table 3 for the correct OPTISEP® filter module part numbers.

Table 3. OPTISEP Ordering Information

Description	Part Number	Regenerated Cellulose 100K
OPTISEP 11000 holder	70-900-2300	
OPTISEP 11000 filter module 1.5 mm channel 100 ft² (9.8 m²)		74-J5B -0100
OPTISEP 11000 filter module 1.5 mm channel 50 ft² (4.9 m²)		72-J5B -0100
OPTISEP 11000 filter module 1.5 mm channel 10 ft² (0.9 m²)		71-J5B -0100
Cart for OPTISEP 11000 holder	0050-53-02	

NCSRT

NCSRT, Inc.
1000 Goodworth Dr.
Apex, NC 27539
USA

Phone: +1 919 387 8460
Fax: +1 919 387 8540
email: info@ncsrt.com
Web: http://www.ncsrt.com