Isolation of small molecules with *SmartFlow*™ TFF

This *Isolation of small molecules* protocol is intended for isolating a small molecule from a fermentation broth. This process has been repeatedly implemented with consistent success in *E.coli* and *Pichia* fermentations with products under 5,000 molecular weight. This process works best with secreted target molecules, but acceptable results will be obtained with lysed cell starting material.

The initial step isolates the target molecule from the fermentation broth by using a 10 or 30 kD regenerated cellulose 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 to 5X prior to starting the diafiltration.

Application: Isolation of Small Molecules protocol:

Process Conditions:
Product: Small molecule < 5 kD MW
Process Objective: Isolation from fermentation broth with a batch size ranging from 100-1000L.
Procedure: Concentrate the starting material 5X and perform a 3X diafiltration
Isolation Loop Filter: OPTISEP® 11000 Regenerated Cellulose UF membrane, 0.75 mm channel height
Isolation Loop Velocity: 100 cm/sec
Expected Yield: >95% product yield

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

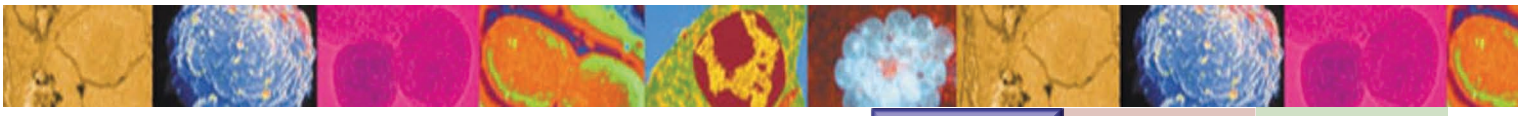
Table 1 Membrane area determination – isolation loop

	A	B	C	D	E		
	Starting Volume (liters)	LM* for isolation step	Membrane area required (Col A/ Col B)	OPTISEP 11000 filter module (9.8 m²) 0.75 channel height	Velocity of retentate at the membrane surface (cm/sec)	Shear sec^{-1}	Recirculation flow rate (per 9.8 m² OPTISEP 11000 module)
Production		60		RC 30 kD 74-E5B-0030 RC 10 kD 74-E5B-0010	100	6,470	260 L/min (70 gpm)

* L starting material/ m² membrane area

The isolation step uses the OPTISEP 11000 module with RC 30 kD or 10 kD 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 260 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 then be set to 40 psi. The resulting inlet pressure should be around 50 psi, which results in a TMP of around 45 psi. As the pressure increases, the recirculation rate will decrease due to pump slip. Adjust the pump speed to maintain the desired recirculation rate as the pressure is adjusted.

Table 2 - Membrane recommendations

Target Molecule	Membrane	Minimum Pore size
Protein < 5 kD	RC	30 kD
Carbohydrate ≤ 1 kD	RC	10 kD
Carbohydrate (greater than 1 kD and less than 5 kD)	RC	30 kD
Organic Acids (i.e. Amino)	RC	10 kD

Collect the permeate of the isolation step in the recirculation reservoir for the concentration step.

When the isolation step reaches 5X concentration, start the diafiltration to increase the yield of the target product. After the 3X diafiltration is complete, the isolation loop may be cleaned. Concentrate the product in the permeate tank to the desired level. Refer to the NCSRT Ultrafiltration, concentration and diafiltration protocol to concentrate the product to the desired levels and have it in the correct buffer for subsequent processing.

For small scale verification of the Isolation of small molecules protocol prior to scale up Table 3 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 process will require a permeate holding tank with a minimum volume of 90 L.

Table 3 - Small scale protocol requirements

	Starting Volume (liters)	LM for isolation step	RC 10 Membrane area required (Col A/ Col B)	OPTISEP 11000 filter module (10 ft² (0.9 m²)) RC 10 kD 0.75 gasket	Velocity of retentate at the membrane surface (cm/sec)	Shear sec^{-1}	Recirculation flow rate	TMP
Isolation Step	60	60	1.0	71-E5B-0010	100	6,470	30.7 l/min (8.1 gpm)	45

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 small molecules WORKS*™ Optimization Procedure from NCSRT.

To learn how others have applied the patented *SmartFlow* filter modules technology to their separations, consult the *Isolation of small molecules* Case Study.

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