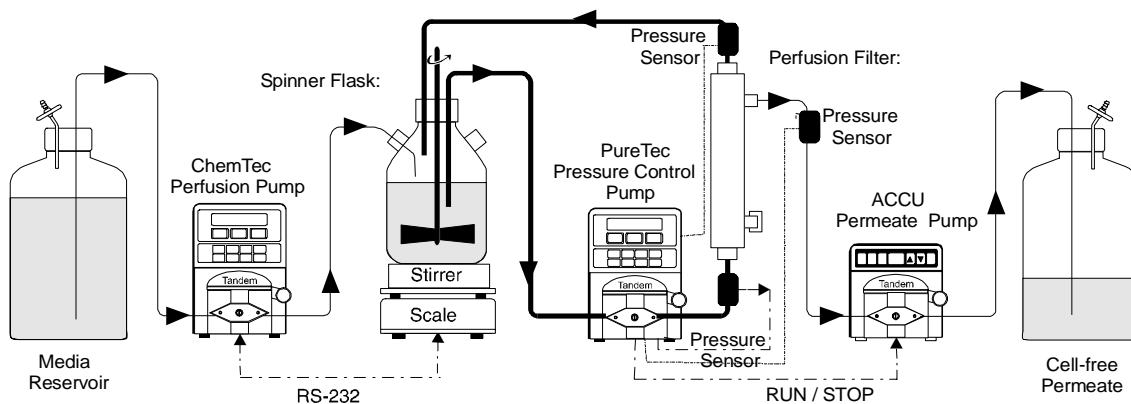


## Continuous Harvesting of Extra-Cellular Proteins from Mammalian Cell Cultures Utilizing Small-Scale Perfusion

### SUMMARY:

The use of a small-scale cell culture system not only maximizes the production of valuable protein but also avoids the high capital cost associated with system scale-up. Continuous harvesting can provide significant improvements in protein yields when compared to those of typical batch operations. For example, Brennan et al (1) reported a 27-fold higher level of monoclonal antibody in a 48-day perfusion culture of hybridoma cells (800µg/ml versus 30µg/ml in a 7-day batch culture).



### FEATURES:

Reactor level problems typically associated with level probes and sensors are avoided by scale feedback to the ChemTec. In the small-scale continuous perfusion system shown above, a ChemTec maintains the media level (constant weight) of a Spinner Flask during perfusion. An autoclavable filtration cartridge was used to generate cell-free permeate.

The ChemTec automatically meters fresh media into the bioreactor in response to the continuous removal of cell-free permeate containing the expressed protein. The ChemTec maintains a constant bioreactor weight over many days and weeks of continuous perfusion. The ChemTec displays and documents the reactor weight as well as the volume of media added to the reactor. Three (3) different alarms can be activated. For example, the reactor weight alarm is turned on when the set-point weight cannot be maintained (i.e. the media reservoir has run empty).

In the Perfusion System, the PureTec functions as an automated re-circulation pump that also controls the operation of the ACCU permeate pump. The PureTec senses a filter plug-up condition by continuously monitoring the filter back-pressure in three locations. The PureTec monitors the feed pressure (P1), the retentate pressure (P2) and the permeate pressure (P3), and calculates the Trans-Membrane Pressure (TMP).

- (1) Brennan, A.J., J. Shevitz and J.D. MacMillan. 1987. A perfusion system for antibody production by shear sensitive hybridoma cells in a stirred reactor. New Brunswick Scientific. Edison, NJ
- (2) Kieron Walsh, Private communication, A/G Technology Corporation.

OVER

## ADDITIONAL FEATURES:


The PureTec controls the Perfusion process in Constant Rate or Constant Pressure Mode. The PureTec has several independent alarms that can be set to Stop and alarm as soon as their limits are exceeded, and simultaneously stop the ACCU permeate pump. Typically, in Constant Rate mode, this occurs when a user-definable High Pressure limit is exceeded, and in Constant Pressure Mode, upon dropping below a user-defined Low Flow limit.

The PureTec pump delivers the suspended cells at a low shear rate, typically 1000-2000 sec<sup>-1</sup>, through a hollow fiber micro filtration cartridge and returns the cells back to the Spinner Flask. The cell-free permeate, containing the expressed protein, is perfused with an ACCU pump at a rate that is measured in bioreactor volumes per day (BVD). The BVD should be optimal for cell viability, cell density and / or production of protein (i.e. 0.5 –2.0 BVD).

In a typical application, the required micro filter surface area is one (1) square foot for every 50 liters of perfused media (2). For example, in a 3-liter bioreactor, perfusion volumes may range from 1.5 – 6.0 liters per day over the course of 30 days or a total 45 -180 liters. Thus the perfused volume requires a filter surface area ranging from 0.9 sq. ft. to 3.6 sq. ft.

## DOCUMENTATION:

The data output of the ChemTec and PureTec can be sent to a printer, or archived to a PC using the SciDoc Documentation Package that automatically populates a Custom Excel Spreadsheet that records the data and automatically populates charts. An example appears below:

											Operator	KS
<small>8/10/3; 11:39 PURE2.02; Constant Rate; P-Source; P1; CW; Tubing=16; Alarms:CV=1;RT=1;LP=1;HP=1;FW=2; Limits; CV=1000.0; RT=01.00; LP= 0.0; HP=30.0; FW=1000.0</small>											Date	08/10/03
<input type="radio"/> Concentration											Time	11:30 am
<input type="radio"/> Diafiltration											Product Batch Number	123
<input type="radio"/> Init. Process Vol.											Product Description	Sample
<input type="radio"/> Clear Data											Conditions	20 C
<input type="radio"/> 400											Membrane Manufacturer	Pall
											Membrane Type	Minimate
											Membrane Lot Number	123456
											Membrane Serial Number	987654
											Membrane Pore Size	100K Omega
											Membrane Surface Area, sq M	0.0050
MT	CV	FQ	P1	P2	P3	TM	FF	FP	FR	ST	AL	
11:39:52	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	0.0	START		
11:40:29	25.0	11.2	15.1	0.7	0.0	7.9	50.0	21.2	28.8	RUN		
11:40:59	50.0	22.7	15.3	0.8	0.0	8.0	50.0	22.8	27.2	RUN		
11:41:29	75.0	34.8	15.3	0.8	0.0	8.0	50.0	22.0	28.0	RUN		
11:41:59	100.0	46.5	15.3	0.8	0.0	8.1	50.0	23.2	26.8	RUN		
11:42:29	125.0	58.0	15.2	0.8	0.0	8.0	50.0	22.8	27.2	RUN		
11:42:59	150.0	70.0	15.3	0.8	0.0	8.1	50.0	23.2	26.8	RUN		
11:43:29	175.0	81.4	15.2	0.8	0.0	8.0	50.0	24.0	26.0	RUN		
11:43:59	200.0	93.4	15.4	0.8	0.0	8.1	50.0	24.8	25.2	RUN		
11:44:29	225.0	105.1	15.4	0.9	0.0	8.1	50.0	23.6	26.4	RUN		
11:44:59	250.0	116.6	15.3	0.9	0.0	8.1	50.0	23.6	26.4	RUN		
11:45:29	275.0	128.3	15.3	0.9	0.0	8.1	50.0	23.2	26.8	RUN		
11:45:59	300.0	139.8	15.4	0.9	0.0	8.2	50.0	22.8	27.2	RUN		
11:46:29	325.0	151.3	15.4	0.9	0.0	8.2	50.0	23.2	26.8	RUN		
11:46:59	350.0	162.9	15.5	0.9	0.0	8.2	50.0	22.0	28.0	RUN		
11:47:29	375.0	174.4	15.4	0.9	0.0	8.2	50.0	21.6	28.4	RUN		
11:47:59	400.0	185.8	15.4	0.9	0.0	8.2	50.0	23.6	26.4	RUN		
11:48:29	425.0	197.5	15.4	0.9	0.0	8.2	50.0	23.6	26.4	RUN		
11:48:59	450.0	208.7	15.5	1.0	0.0	8.2	50.0	22.8	27.2	RUN		
11:49:42	485.0	231.5	26.9	1.6	0.0	14.2	75.0	34.0	41.0	RUN		
11:50:12	522.5	249.3	28.4	1.6	0.0	15.0	75.0	35.2	39.8	RUN		
11:50:42	560.0	267.0	29.3	1.6	0.0	15.4	75.0	35.2	39.8	RUN		
11:51:12	597.5	284.6	29.8	1.7	0.0	15.7	75.0	34.8	40.2	RUN		
11:51:42	635.0	302.3	29.9	1.7	0.0	15.8	75.0	36.0	39.0	RUN		
11:52:12	672.5	319.9	30.2	1.7	0.0	15.9	75.0	34.8	40.2	RUN		

Several Pump Head / Motor combinations are available, contact SciLog with your specific needs.

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