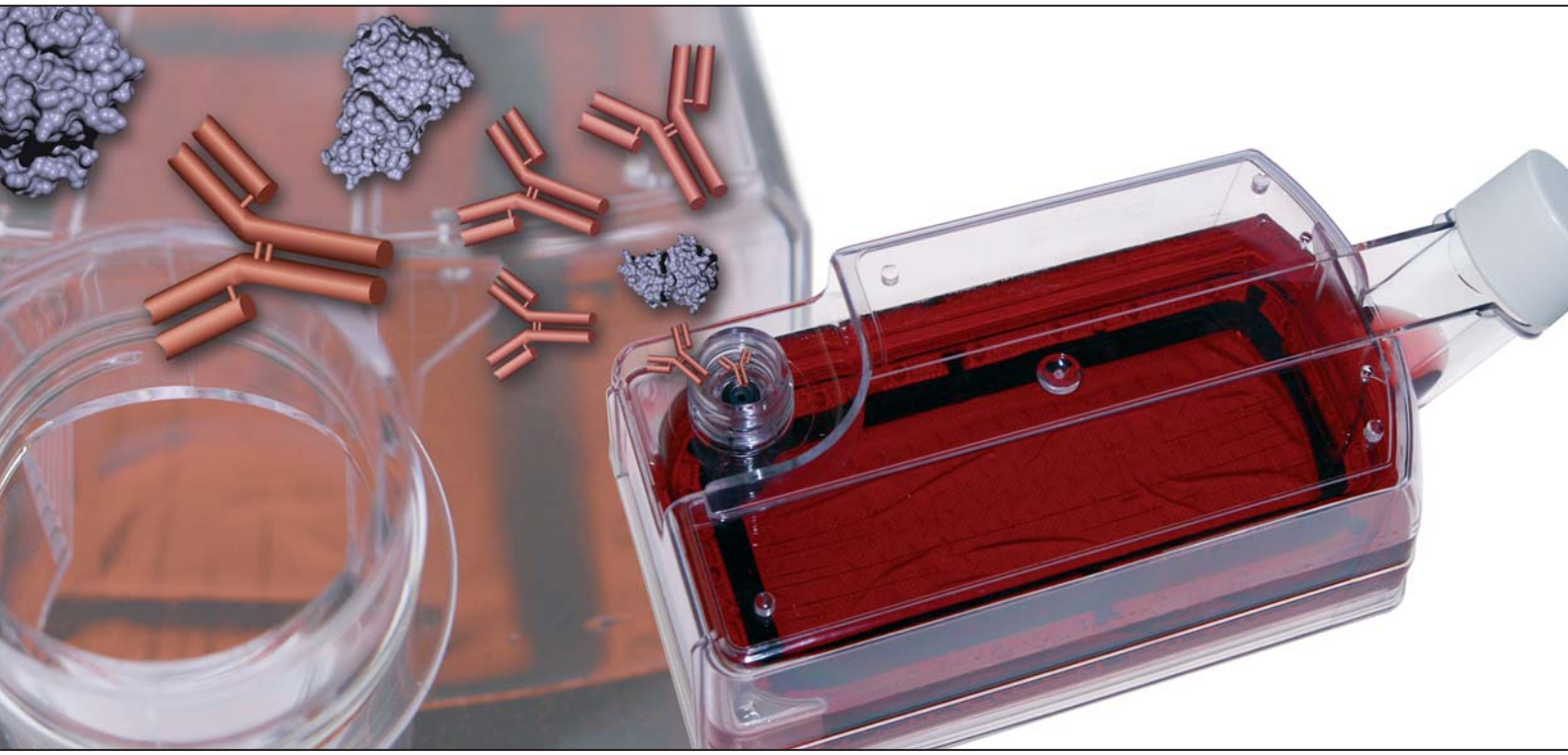
CELLine



Disposable Bioreactor for Efficient Protein Expression

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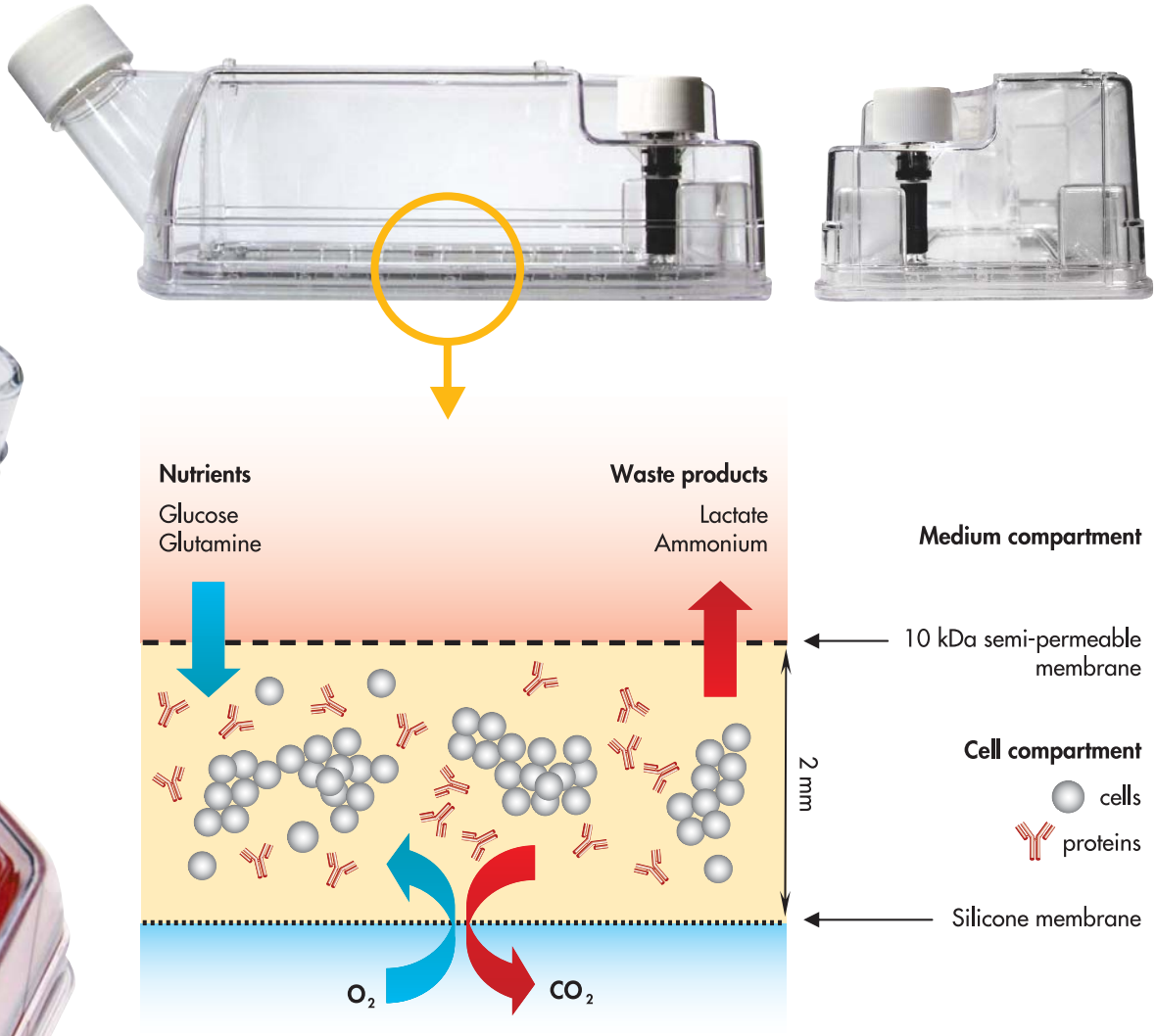
Two-Compartment Technology



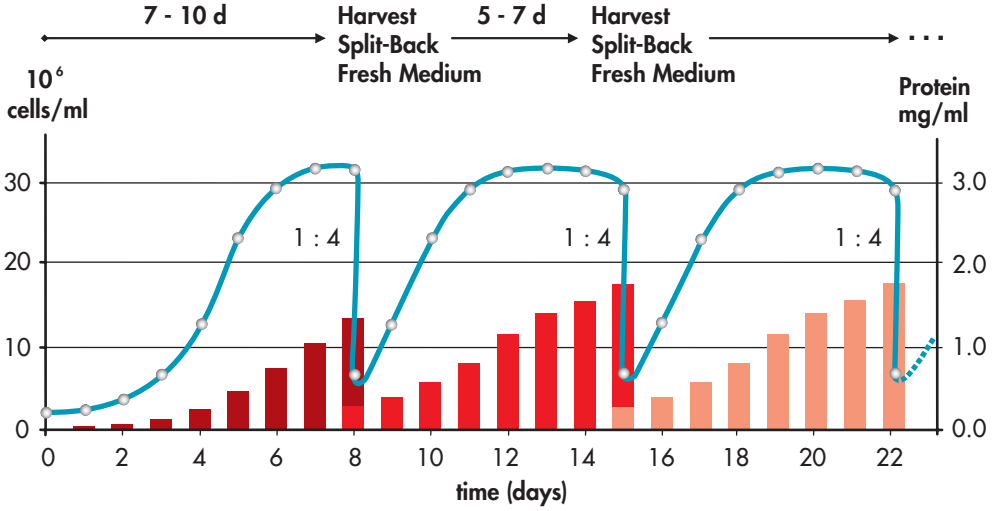
Efficient cell cultivation relies on maintaining an optimal supply of oxygen and nutrients, as well as on removing inhibiting metabolic waste products efficiently. These factors limit the maximum cell density obtainable from standard, homogeneous cell culture techniques which are consequently poor at achieving high expression levels of proteins.

The Two-Compartment Bioreactor CELLine is designed to overcome such limitations. The innovative CELLine technology separates the bioreactor into a medium and cell compartment by means of a 10 kDa semi-permeable membrane. This membrane allows a continuous diffusion of nutrients into the cell compartment with a concurrent removal of any inhibitory waste product. The individual accessibility of the compartments allows to supply cells with fresh medium without mechanically interfering with the culture.
Efficient gas transfer is ensured by a silicone membrane which forms the cell compartment base. This membrane provides an optimal oxygen supply and control of carbon dioxide levels by providing a short diffusion pathway to the cell compartment. All together, the technology built into CELLine allows simulating quasi *in vivo* cultivation conditions leading to high cell densities.

Application

Efficient protein expression

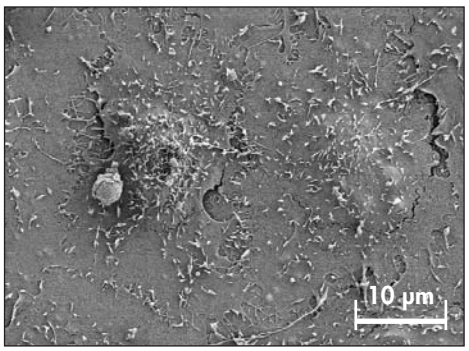
Cells growing under the optimal conditions created in CELLine reach densities of 10^7 to 10^8 cells per ml, a cell concentration that is about two magnitudes higher than the one obtained with conventional culture techniques. Consequently, the concentration of expressed protein is typically 50 to 100 times above what is found in standard cell culture disposables. In addition, CELLine has been designed to maintain cells for several months in culture allowing to periodically harvest expressed proteins. By combining high product concentration with recurring product collection, large amounts of highly concentrated proteins are routinely obtained in CELLine. With classic culture techniques, contaminating proteins originating from serum or cells are a significant part of the total protein fraction. In contrast, due to the high product concentrations obtained with CELLine, the relative level of contaminating proteins to the expressed protein is much lower. In many cases the quality of monoclonal antibodies produced with CELLine is sufficient to perform standard laboratory tests, like Western Blot, without the need of any further purification steps.

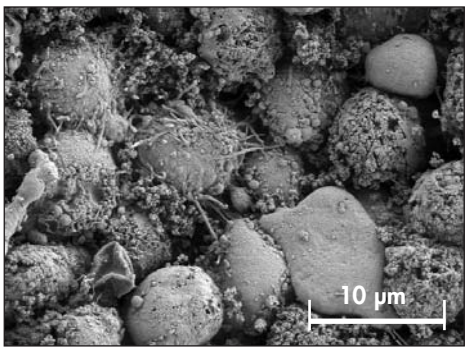


The choice between **CELLine** *classic* and **CELLine** *adhere* makes it possible to grow both suspension or anchorage-dependent cells, hence allowing production of different biomolecules using different expression systems such as monoclonal antibodies in hybridomas, recombinant proteins in transfected cell lines and virus particles in packaging cells.

***In vivo*-type cultivation**

Successful cultivation of different cell types demands not only skillful handling, but also depends to a large extent on the chosen cultivation system. CELLine technology closely mimics the physiological conditions within the body enabling production of drastically increased cell densities and more organotypic cell morphologies, sometimes even to the extent of 3-dimensional cell growth.





Representative EM micrographs showing the flat morphology of HEp-2 cells growing as monolayer in a standard T-Flask (left) compared to the rounded shape of the same cells when cultivated in CELLine *adhere* (right).
(With courtesy of W. Pfaller, Institute of Physiology, University of Innsbruck)

Further Reading:

Efficient laboratory-scale production of monoclonal antibodies using membrane-based high-density cell culture technology. Trebak *et al.* (1999) J. Immunol. Methods, 230: 59-70

Long-Term High Level Protein Expression in Adherent, Protein-free Growing BHK Cells Using INTEGRA CELLine *adhere* 1000 Bioreactor Flasks. J. Mittermaier and M. O. Zang-Gandor (2004) Genetic Engineering News, 24(12): 42

For more references go to www.integra-biosciences.com or contact us at cell@integra-biosciences.com.

Easy and economical protein expression

Easy operation

Due to its uncomplicated design working with the CELLine bioreactor is as simple as with any standard tissue culture flask. For a straightforward control of the growth process, microscopic observation of the cells is made possible by the transparent design of the CELLine *classic* bioreactor. The system operates independently of any complicated control technology and works without any pump systems or agitation devices. Easily and securely stackable, CELLine flasks occupy a minimum of space in any standard CO_2 incubator. A specific adaptation of the cell culture techniques or media composition is generally not necessary when starting to work with CELLine and both, serum-supplemented or serum free media are suitable.

Cost efficiency

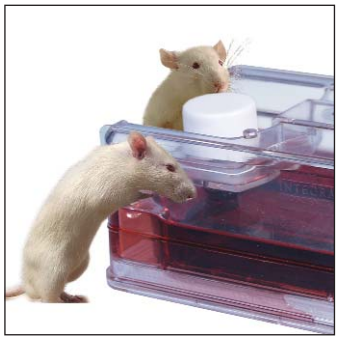
CELLine has been designed to bring substantial cost savings to cell cultivation. Labour costs are considerably reduced, because fewer disposable flasks need to be handled compared to other culture techniques in order to produce milligram amounts of protein. Also the expenses for media supplements are significantly reduced, since the addition of serum or other synthetic additives can be limited to the cell compartment. Furthermore, the high production yield and quality obtained in the relatively small culture volume of CELLine contribute to reduce costs and labour time in the subsequent downstream processing steps of the product.

Economic analysis for mAb Production

	Units	CELLine 1000		T-Flask 225cm²	
Productivity					
Hybridoma productivity (literature value)	pg/h x cell	0.3		0.3	
Cell density	cell/ml	$3x10^7$		$1x10^6$	
Culture volume	ml	20		50	
Yield per harvest (7 days)	mg	30.24		2.52	
mAb concentration	mg/ml	1.51		0.05	
Production Costs (250 mg mAb)		Amount	Cost (in $)	Amount	Cost (in $)
Harvests per disposable		8		1	
Number of disposables		1	150	100	300
Medium ($ 20 per liter)	litres	8	160	5	100
Serum consumption* ($ 300 per liter)	ml	16	4.8	500	150
Labour** ($ 25 per hour)	min	120	50	500	208
Total costs			**364.8**		**758**
Costs per mg mAb			**1.46**		**3.03**

* The medium is supplemented with 10% of serum. In the case of CELLine, only the medium in the cell compartment is supplemented.

** Labour is calculated as the time used for inoculating and harvesting one CELLine flask (15 min) or one T-Flask (5 min) multiplied by the number of harvests or flasks, respectively.



Animal welfare

CELLine is a disposable bioreactor that is competitive in costs and performance to the production of monoclonal antibodies using mice ascites. As an added benefit, when expressing monoclonal antibodies in hybridomas using CELLine, the antibody preparation is free of any contamination from mouse immunoglobulins. Over recent years, CELLine technology has been successfully adopted worldwide for the production of monoclonal antibodies and thereby has contributed diminishing the use of laboratory mice.

Bioreactor Types

CELLine *classic*

CELLine *classic* (CL) is ideal for laboratory scale applications using suspension cells or adherent cells in combination with microcarriers. The unit is optimised for cultivation of hybridomas and many other cell types (e.g. CHO, NSO, SF cells).

CELLine *adhere*

CELLine *adhere* (AD) is specifically adapted to allow growth of anchorage-dependent cells (e.g. HEK, BHK, CHO cells). The bioreactor contains a woven, polyethylene terephtalate (PET) matrix in the cell compartment providing an ideal surface for cell attachement.

PET matrix inlay of CELLine *adhere*

Technical Specifications

CELLine Two-Compartment Disposable Bioreactors are manufactured from optically clear virgin polysterene with a gas transfer bottom made of a molded silicone membrane providing a 0.2 µm vent barrier. The compartments are separated by a 10 kDa semi-permeable cellulose acetate membrane and individually pressure tested for integrity. The bioreactors are easily stackable owing to specific stabilisation interlocks, packed individually in easy to open medical-grade blister packaging, sterilised by gamma irradiation and non-pyrogenic.

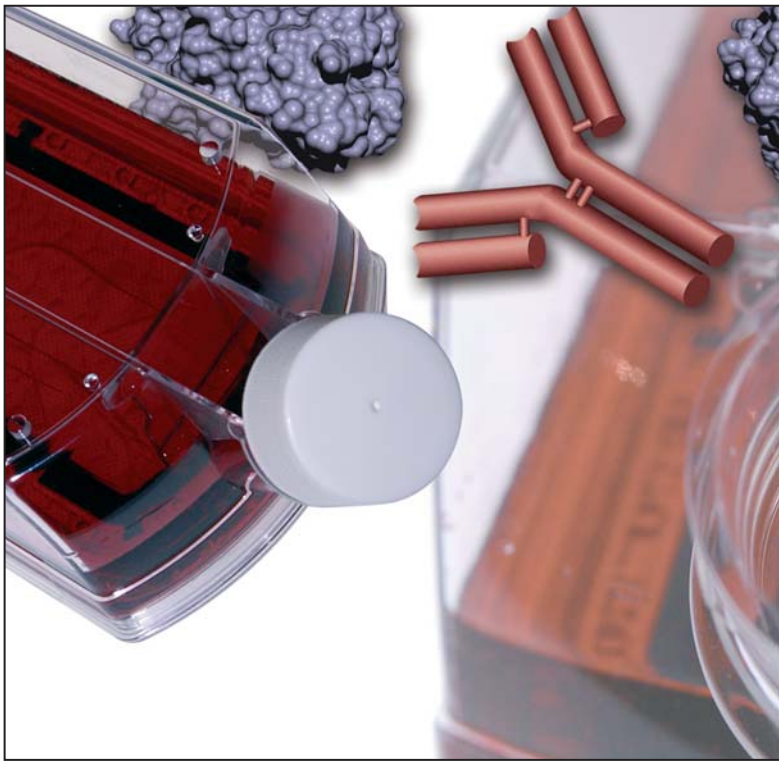
	CELLine CL 350	**CELLine CL 1000**	**CELLine AD 1000**
			
Size: L x W x H (mm)	190 x 95 x 62	275 x 120 x 80	275 x 120 x 80
Weight (g)	185	334	336
Medium compartment cap	28 mm vented (0.2 µm), green polypropylene cap with polypropylene liner	38 mm vented (0.2 µm), white polypropylene cap with polypropylene liner	38 mm vented (0.2 µm), black polypropylene cap with polypropylene liner
Cell compartment cap	24 mm polypropylene cap with polyethylene liner	28 mm polypropylene cap with polyethylene liner	28 mm polypropylene cap with polyethylene liner
Cell compartment inlay	none	none	PET matrix inlay
Microscopic viewing (inverted microscope)	center window requires objectice working distances of 2.5 mm	center window requires objectice working distances of 2.5 mm	limited visibility due to inlay matrix
Vertical and horizontal volume markings	50 - 350 ml	100 - 1000 ml	100 - 1000 ml

Ordering Information

Product Name	**Description**	**Quantity/Case**	**Item No.**
CELLine CL 350	Disposable Two-Compartment Bioreactor for suspension cells, 350 ml media volume, 5 ml culture volume	5	90010
CELLine CL 1000	Disposable Two-Compartment Bioreactor for suspension cells, 1000 ml media volume, 15 ml culture volume	3	90005
CELLine AD 1000	Disposable Two-Compartment Bioreactor with matrix inlay for anchorage-dependent cells, 1000 ml media volume, 15 ml culture volume	3	90025

Having troubles expressing enough recombinant protein?

Boost your production of monoclonal antibodies or recombinant proteins by cultivating cells at highest densities with CELLine, the disposable bioreactor based on Two-Compartment Technology.



Efficient protein expression

50-100 times higher product concentrations compared to classic cell culture disposables

Easy operation

as simple as using a tissue culture flask

Cost efficient

90% less media supplements and reduced handling time

Applications

Monoclonal antibody production in hybridomas

Recombinant protein expression in transfected cells

Virus production

Continuous culture maintenance for long-term studies

High-density cell culture

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2.3 CELLine AD 1000

2.3.1 Required Material and Preparation

- CELLine AD 1000 Bioreactor
- Standard 25 ml serological pipettes
- Pipetting aid
- Preculture of 25 x 10^6 viable cells
- 1000 ml of fresh medium suitable for your individual cell type and equilibrated to the desired culture temperature (see 3.1).
- 5ml of fresh complete medium (see note 3.2)

For more information on media composition please also refer to general note 3.2.

2.3.2 Equilibration of CELLine

Day 1 In order to obtain optimal performance of CELLine put 25 ml of growth medium into the medium compartment and let the semi-permeable membrane equilibrated for at least 5 minutes (see 3.3).

2.3.3 Preparation of Inoculum

Obtain 25 x 10^6 viable cells from a pre-culture in log growth phase and suspend the cells in 15 ml fresh medium resulting in a minimal concentration of about 1.5 x 10^6 viable cells/ml (see 3.4).

2.3.4 Inoculation of CELLine

Loosen the black cap of medium compartment in order to prevent air lock. Aspirate the 15 ml cell suspension into a serological pipette, open the cell compartment and inoculate the cell compartment by inserting the pipette into the black silicone cone.

It is important to minimize the introduction of air bubbles into the cell compartment during seeding. In case air gets trapped within the cell compartment try to carefully remove the big bubbles by carefully drawing them back into the pipette together with fluid. Close the cell compartment by completely tighten the cap.

After seeding add 975 ml of equilibrated medium into the medium compartment and then completely close both caps. Place the CELLine into a standard CO_2 incubator under culture conditions appropriate for your individual cell type.

2.3.5 Culture monitoring (optional)

Day 3 After 72 hours, take a sample from the cell compartment for assessment of cell density and viability, expression levels of recombinant protein or determination of other individual critical culture parameters. This is especially important when culturing a new cell type in order to establish a working protocol.

2.3.6 Cell compartment harvest and medium change

Day 10 In general, the first harvest is recommended 10 days after inoculation (see note 3.5).

In order to harvest the cells, simply pour off and discard all medium from the medium compartment.

Avoid to shake the CELLine during this process (see note 3.6)

Gently harvest all liquid from the cell compartment by aspirating content with a 25 ml serological pipette (Do not force the liquid out of the cell compartment). The cell compartment will comprise about 15 ml cell suspension and the individual secreted product. Due to osmotic flux of liquid from the medium - to the cell compartment the total volume might be slightly increased (see note 3.7).

Gently add 15 ml of fresh, preheated complete medium into the cell compartment.

Add 1000 ml of fresh, preheated nutrient medium to the medium compartment. Place CELLine back into the incubator until next harvest.

2.3.7 Harvesting Cycles

from Day 17 Consecutive harvests can approximately be made every 7 days (depending on the individual application and cell type used). All harvests are performed as outlined above and should include a change of the culture media.

Periodically, cells can be monitored for growth and production by removing a small sample from the cell compartment.

If the CELLine Bioreactor is handled with care and the sterility barrier is not broken individual cultures can be maintained over several months.