WHEATON®

CELLine™ Bioreactor Flask

A High-Throughput Static Culture Flask
As An Alternative to Shake Flask Culture

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A High-Throughput Static Culture Flask As An Alternative to Shake Flask Culture

Experiment Overview

INTRODUCTION

Irvine Scientific specializes in developing and manufacturing cell culture media for bioproduction. To ensure the utility of the medium, CHO cell lines are tested for density, viability and productivity. The cell lines are tested at the small scale in conventional shake flasks. Gram level production of proteins and antibodies can be laborious using this type of *in vitro* method. Irvine Scientific investigated a new membrane flask technology to increase small scale production levels and to decrease culture maintenance requirements.

ABSTRACT

Discovery scale, high-throughput methods for *in vitro* antibody and protein production can be timely and typically requires fixed equipment such as shakers, pumps, or stir tank reactors. This experiment evaluated membrane flask technology that allows for high-density cell culture as well as concentration of the expressed proteins or antibodies. Two bioreactor flasks, the CELLine 350 and the CELLine 1000, were evaluated and compared to shake flask experimental controls. The CELLine 350 has a 350mL media compartment with a 5mL cell compartment, and the CELLine 1000 has a 1000mL media compartment with a 15mL cell compartment. The cell compartments are created by a gas permeable bottom membrane and an upper dialysis membrane allowing for diffusion of media into the cell compartment. The testing of both CELLine sizes allowed for a comparison of scalability.

This experiment also evaluated two different media formulations. Both the shake flask control and the CELLine bioreactor flasks utilized either BalanCD™ CHO Growth A or IS CHO-CD G10.7 media. The shake flask cultures were operated as both batch and fed-batch cultures (fed on days 3-6). The CELLine bioreactor flasks had IgG harvested, the media changed and the cells split every 7 days. Cell concentrations, viability and IgG quantification were measured across the 8 assessments. (2 CELLine 350s, 2 CELLine 1000s and 4 – 125mL shake flask cultures).

How Do CELLine Bioreactor Flasks Work?

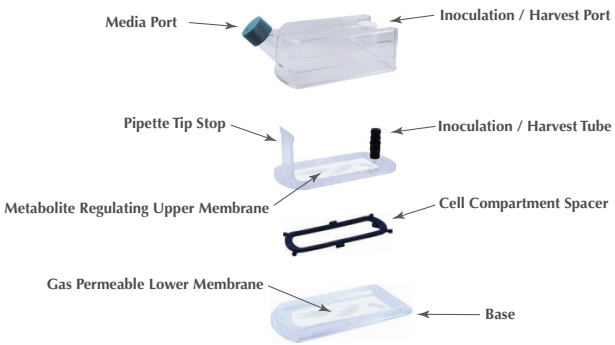
CELLine bioreactor flasks utilize a dual membrane system to create an optimal cell chamber. The chamber resides at the bottom of the flask and is formed by a gas permeable bottom membrane and a top dialysis membrane. The media compartment is accessed via the green vented cap. The cell compartment is accessed via the white cap. The cell compartment cap gives way to a pipette septum. This septum connects directly to the cell compartment for simple seeding and harvest.

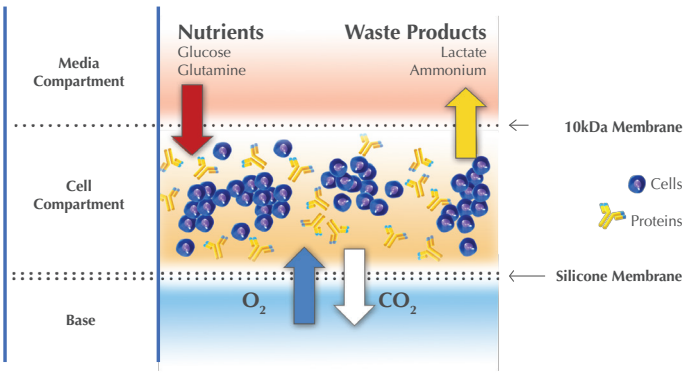
Media Compartment — The media compartment allows for bulk storage of cell culture growth medium. This reduces the medium refreshing requirement significantly as the media compartment is fifty times the size of the cell compartment.

Metabolite Regulating Upper Membrane — The upper dialysis membrane has a 10 kDa cut off limit. This regulates the flow of metabolites to and from the cell compartment and retains all proteins in the cell compartment.

Cell Compartment — The cell compartment provides the ideal area to inoculate and achieve high density cultures. The compartment concentrates cells, their products, and limits the requirement for any exogenous growth factors.

Gas Permeable Lower Membrane — With static cultures, gas transfer rates can be the limiting factor in high density cultures. The CELLine bioreactor flask places the cells directly against the gas permeable membrane to achieve optimal levels of oxygen and carbon dioxide.

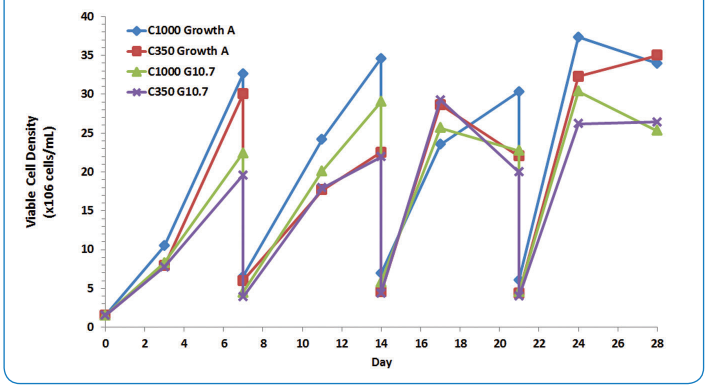




RESULTS

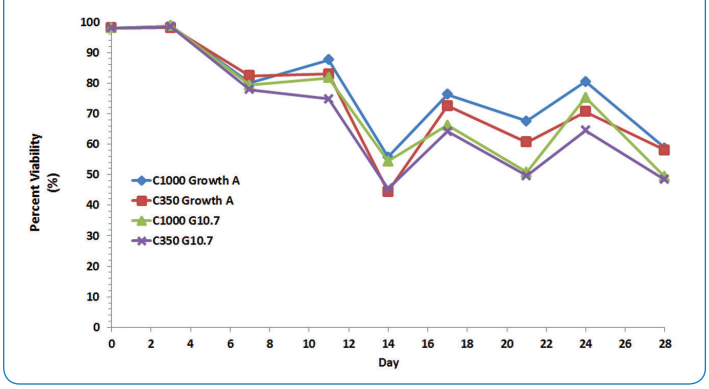
CELLine Bioreactor Cell Culture

Figure 1A



Cell concentrations were measured every three to four days in the CELLine bioreactor flasks. All four iterations were seeded with the same cell concentrations (1.5 x 10^6 cells/mL). Cells were harvested, diluted 1:4 and reintroduced into the flask every 7 days. Viable cell concentrations reached above 35 million cells/mL in each flask. This is approximately 525 million cells in the CELLine 1000 flask and 175 Million cells in the CELLine 350. At term, the CELLine bioreactors utilizing the BalanCD™ CHO Growth A medium maintained the highest cell concentrations.

Figure 1B



Percent viability of cells in the CELLine bioreactor flasks were measured every three to four days for 28 days. The viability slowly decreased over time; however, the concentration of IgG was not affected. In the CELLine bioreactors, BalanCD™ CHO Growth A maintained the highest cell viability throughout the experiment.

Antibody Production in CELLine Bioreactor Flask

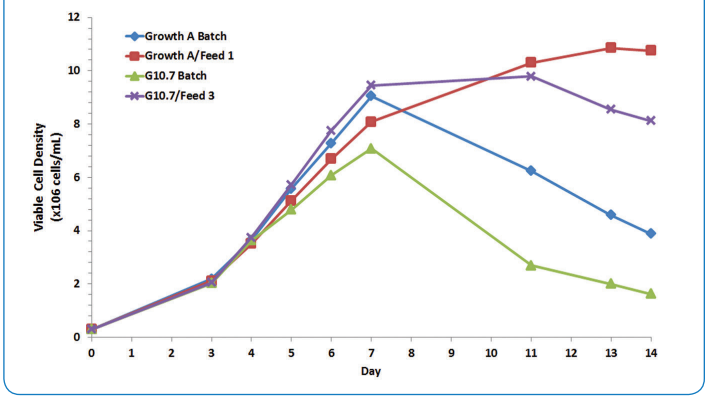
Condition \ Day	Concentration g/L								Total Product Harvest	Final Yield
	3	7	11	14	17	21	24	28	g/mL	Grams
C1000 Growth A	0.84	6.93	6.38	9.65	3.77	8.22	5.04	8.80	33.60 / 60	0.50
C350 Growth A	0.81	6.95	7.37	9.82	4.69	8.21	4.77	8.73	33.71 / 20	0.17
C1000 G10.7	0.96	6.68	6.85	9.62	4.17	7.61	5.17	7.54	31.45 / 60	0.47
C350 G10.7	0.92	6.50	6.92	9.53	4.48	7.54	4.52	6.89	30.46 / 20	0.15

Highlighted column indicates harvest

The IgG concentration was measured throughout the CELLine bioreactor flask operation. A total of 60mL was collected from each CELLine 1000 over the course of 4 harvests from the 15mL cell compartment. A total of 20mL was collected from the CELLine 350 over the course of 4 harvests from the 5mL cell compartment. The results show up to 0.5 g IgG production in 28 days and indicate linear scaling of the CELLine 1000 and CELLine 350 devices.

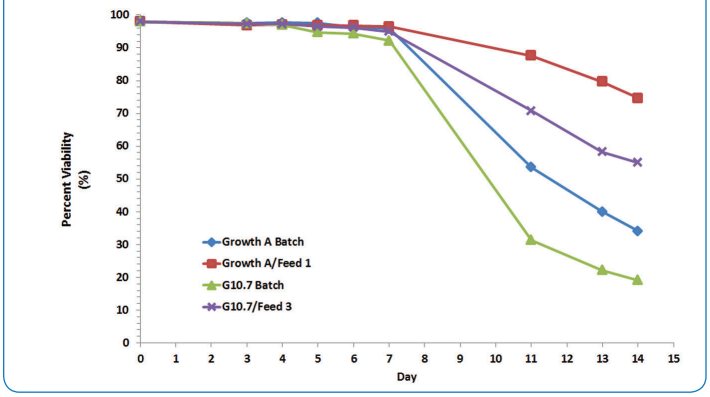
Shake Flasks Cell Culture

Figure 1C



Shake flask cultures were monitored for viable cell density over the course of 14 days. Viable cell density peaked with the fed-batch flask utilizing BalanCD™ CHO Growth A and the BalanCD™ CHO Feed 1 above 10 million cells/mL. Both fed-batch cultures maintained higher cell concentrations than the batch shake flask cultures.

Figure 1D



Percent viability of the cells in the shake flask cultures were monitored over the 14 day experiment. Fed-batch cultures maintained higher percent viability than batch cultures and were above 50% at term.

Antibody Production in Shake Flask

Condition	Day				Total Product Harvest	Total Yield
	3	7	11	14	g/mL	Grams
Growth A Batch	0.15	1.18	1.52	1.63	1.63 / 30	0.049
Growth A / Feed 1	0.15	1.12	2.38	2.77	2.77 / 36	0.100
G10.7 Batch	0.16	1.05	1.29	1.36	1.36 / 30	0.041
G10.7 / Feed 3	0.16	1.29	2.67	2.78	2.78 / 36	0.100

Highlighted column indicates harvest

The shake flask IgG concentration was monitored during culture for both batch and fed-batch conditions.

CELLine™ Bioreactor Flask

Materials and Methods

Shake Flask Culture

Shake flasks (125mL) were used in conjunction with BalanCD™ CHO Growth A or IS CHO-CD G10.7, 30mL initial working volume. Five percent feed of BalanCD™ CHO Feed 1 or BalanCD™ CHO Feed 3, was provided on days 3, 4, 5 and 6 for a total of 20% initial volume. Conditions: shake speed 120 RPM, 5% CO_2, and 37°C humidified incubator. Cells were counted with Beckman Coulter Vi-Cell and IgG quantified by Forte' Bio Octet kEQ. Initial seeding density was 0.3 x 10^6 cells/mL.

CELLine Bioreactor Flask Culture

CELLine bioreactor flasks were used in conjunction with BalanCD™ CHO Growth A or IS CHO-CD G10.7. Cells were split 1:4 every 7 days. Media was refreshed every seven days. Conditions: 5% CO_2, and 37°C humidified incubator. Cells were counted with Beckman Coulter Vi-Cell and IgG quantified by Forte' Bio Octet kEQ. Initial seeding density was 1.5 x 10^6 cells/mL.

Experimental Design

Experimental – CELLine

Media Tested

- BalanCD™ CHO Growth A
- IS CHO-CD G10.7

Vessels Used

- 350 CELLine (350mL)
- 1000 CELLine (1000mL)

Seeding Density

- 1.5 x 10^6 cells/mL

Culture Protocols

- 28 days
- Cell count & IgG quantification every 3-4 days
- Complete media refreshed every 7 days, split cells 1:4

Control – Shake Flask

Media Tested

- BalanCD™ CHO Growth A (-/+) BalanCD™ CHO Feed 1
- IS CHO-CD G10.7 (-/+) BalanCD™ CHO Feed 3

Vessel Used

- 125mL flask (30mL working vol.)

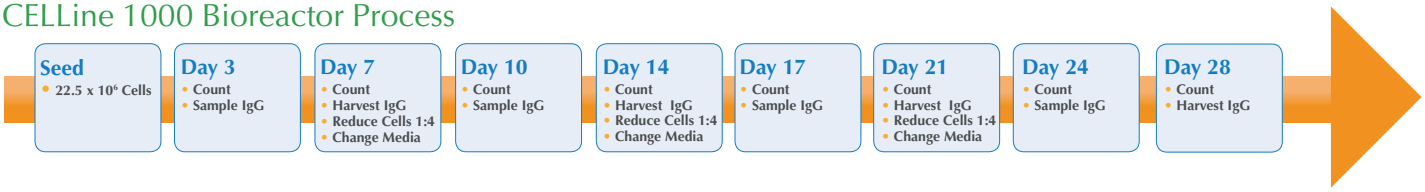
Seeding Density

- 0.3 x 10^6 cells/mL

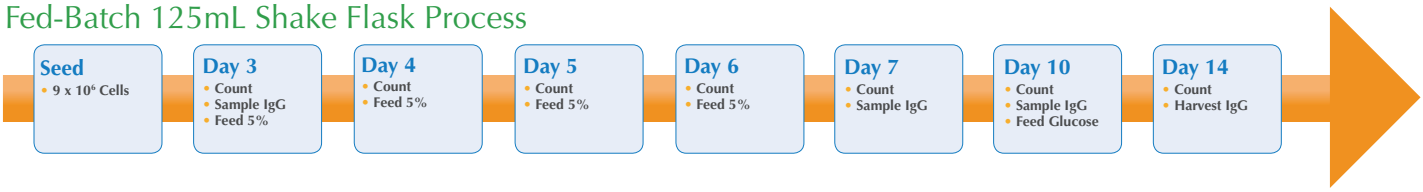
Culture Protocols

- 14 days
- Cell count daily and IgG quantification @ day 7, 10 and 14
- Feed initial 5% working volume of feed to fed-batch conditions on days 3-6

CELLine 1000 Bioreactor Process



Fed-Batch 125mL Shake Flask Process



Media Information

- BalanCD™ CHO Growth A is a chemically-defined growth medium, containing no animal-derived components. The formulation was developed using Irvine Scientific's Rational Culture Media Design® strategy with three model cell lines, and specifically designed to support growth and productivity in a variety of CHO cells in both batch and fed-batch cultures.
- IS CHO-CD G10.7 is part of Irvine Scientific's CHO Media Development Kit. It is a chemically-defined growth media with no animal derived components that was designed to help control metabolic overflow and support growth and productivity in a variety of CHO cells in both batch and fed-batch cultures.
- BalanCD™ CHO Feed 1 and BalanCD™ CHO Feed 3 are both chemically defined, animal component free and protein-free feed media designed to provide cell culture nutrients for fed-batch applications in conjunction with BalanCD™ CHO Growth A. These feeds have been developed to promote cell growth and MAb production in a variety of CHO cells.

CONCLUSION

The experiment provided consistent results in the shake flasks and the CELLine bioreactor flasks. There was more yield using the BalanCD™ CHO Growth A than the IS CHO-CD G10.7. The amount of labor required to operate the CELLine flask was higher than the shake flask but the yield was much higher. The CELLine flask did not require any additional fixed equipment, such as shaking equipment. The purification burden was lower for the CELLine flask as the total harvest volumes were 60mL and 20mL, over four harvests, that contained approximately 7g of IgG per Liter. The shake flask cultures had a single harvest at 36mL and 30mL with an average concentration of IgG of 2.1g per Liter. One surprising result was the potential reduction in scale up time using the CELLine flasks. To operate a 1 Liter shake flask, four times the amount of cells are required for initial seeding; approximately 90 million compared to 20 million required to seed a CELLine bioreactor flask. This could potentially save 1-2 weeks in scale up time. Further studies need to be conducted to determine if the media needs to be refreshed every 7 days or if the media can support cell growth for a longer period of time. This could potentially decrease the operational costs and service requirements.

Input	CELLine 1000 Bioreactor, 14-Days	1L Shake Flask, 14-Day Fed-Batch
Labor: Cell Culture	High: Media exchange and product harvest every 7 days	Low: Minimum feed work
Media Cost Per 14-Day Culture	High: 2L @ $167	Low: 360mL @ $30
Purification Burden	Low: 30mL to purify	High: 360mL to purify
Equipment Required	Low: CO_2 incubator	High: Incubator, shaker platform
Scale Up Prior to Experiment	Low: 2.25 x 10^7 cells needed	High: 9 x 10^7 cells needed, larger seed train

The experiment also showed that the CELLine bioreactor flask size and run time can be selected based on required yield. This CELLine 1000 is 3 times the size of CELLine 350 and scales linearly.

Seeding And Harvesting Densities	CELLine 350	CELLine 1000
Preculture (Viable Cells)	7.5 x 10^6	22.5 x 10^6
Inoculation Volume (mL)	5	15
Inoculation Concentration (Viable Cells/mL)	1.5 x 10^6	1.5 x 10^6
Harvesting Concentration (Viable Cells/mL)	20-40 x 10^6	20-40 x 10^6
Titer (mg/mL)	1 - 10	1 - 10
Antibody Yield per Month (mg)	20-200	60-600

Bioreactor	Cost of Flask	Media Used In 28 Days	14-Day Yield (Grams)	28-Day Yield (Grams)
CELLine 350	$160	1.4L / $116	0.08	0.16
CELLine 1000	$200	4L / $332	0.25	0.49

Price estimates are based on retail cost.

WHEATON® Company Profile

WHEATON is dedicated to providing quality products and services for the laboratory research, diagnostic packaging, and specialty pharmaceutical industries. For over one hundred years, WHEATON products have been present when the greatest discoveries and advances in science were accomplished. Whether it is scientific research or commercial packaging, the WHEATON brand represents quality, reliability and trust. WHEATON is proud to be there when the scientists and packagers of the world say, "Because it's my life's work ... I trust WHEATON."™

Irvine Scientific Company Profile

Irvine Scientific, a member of JX Holdings group, is a worldwide leader in the design, manufacture and distribution of medical devices, including Industrial Cell Culture, Cytogenetic, Assisted Reproductive Technology (ART) and Specialty Media products. Irvine Scientific is a large scale producer of advanced quality cell culture media for the industrial bioprocess, medical, and diagnostic markets. The company's extensive experience in the design of culture media, compliance with ISO and FDA regulations for class II/III medical devices, and industrial scale manufacturing capacity provides its customers with unique capabilities and support. Irvine Scientific delivers products worldwide to biopharmaceutical industry, research and medical laboratory communities.

Ordering and Contact Information

Description	WHEATON P/N
1 CELLine 350 bioreactor flask & 1 Liter BalanCD™ CHO Growth A Medium	WCL0350CHO-1L N/A
1 CELLine 1000 bioreactor flask & 3 Liters BalanCD™ CHO Growth A Medium	WCL1000CHO-3L N/A
1 Liter of Irvine Scientific BalanCD™ CHO Growth A Medium	WCLCHOA-1L
CELLine 350 Flask for suspension cultures (one pack / five pack)	WCL0350-1 / WCL0350-5
CELLine 1000 Flask for suspension cultures (one pack / three pack)	WCL1000-1 / WCL1000-3
CELLine 1000AD for adherent cultures (one pack / three pack)*	WCL1000AD-1 / WCL1000AD-3

*A different media formulation may be required for anchorage dependent cultures.

For technical support or ordering information, email CELLine@wheaton.com.

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