BalanCD CHO DG44

Catalog #	Product	Format	Available Package Sizes*
91177	BalanCD CHO DG44	Liquid	1 L
94148	BalanCD CHO DG44	Powder	10 L

* Additional package sizes are available upon request

Intended Use

For further manufacturing use only

Product Description

BalanCD CHO DG44 is a chemically defined growth medium designed specifically for the growth and productivity of suspension CHO DG44 cell lines. BalanCD CHO DG44 is provided without L-Glutamine to extend shelf life. The recommended L-glutamine concentration to add is 4-8 mM. Glucose should be monitored during culture as additional supplementation may be required. The medium does not contain antibiotics, antimycotics, protein hydrolysates or any other undefined components.

Shipping

Product is shipped at ambient. Upon receipt, store immediately at 2-8 ºC.

Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis, which is available at www.irvinesci.com or upon request.

Storage Instructions and Stability

POWDER MEDIUM

Store at 2-8 °C protected from atmospheric moisture. This product is hygroscopic. Bring container to room temperature before opening and re-seal tightly after opening. When properly stored, unopened powder medium (Catalog #94148) is stable for 24 months. Do not use after the assigned expiration date. The powder should be free flowing; do not use if it is caked. Avoid opening and closing the container multiple times.

LIQUID MEDIUM

Handle using aseptic techniques to avoid contamination. Store at 2-8 °C in the dark. Do not use any bottle of medium that shows evidence of particulate matter or cloudiness. When properly stored, unopened liquid medium (Catalog #91177) is stable for 12 months. Do not use after the assigned expiration date.

Directions for Use

Hydration from Powder Medium

1. Add powder medium Catalog #94148 (25.16 g/L) to desired final volume of WFI (Catalog #9309) into an appropriately sized container.
2. Mix the solution for approximately 30-60 minutes or until the powder is well dissolved (the solution may still appear cloudy at this point).
3. Add 2.20 g/L Sodium Bicarbonate to the solution and mix at moderate speed until completely dissolved.
4. Measure pH. If necessary, adjust pH to 7.0-7.4 with 5N HCl or NaOH.
5. Measure osmolality. Final osmolality is expected to be 285-310 mOsm/kg.
6. Sterile filter through a 0.2 µm filter membrane.
7. Store at 2-8 °C, in the dark for up to 1 year. Avoid prolonged exposure at ambient temperature prior to use.

Medium Supplementation

This medium can be supplemented with L-glutamine by aseptically adding 20-40 mL/L of 200 mM L-glutamine (Catalog #9317) to reach 4-8 mM final concentration prior to use. BalanCD CHO DG44 is formulated to promote the growth of DG44 cell lines which have reintroduced the DHFR gene during cell line development. To promote growth of parental DG44 cells, lacking the DHFR gene, the medium must be supplemented with thymidine at an appropriate concentration.

Adaptation

I. Direct Adaptation to BalanCD CHO DG44

In most cases, CHO cells may be subcultured from a serum-supplemented medium directly into BalanCD CHO DG44.

1. Dispense BalanCD CHO DG44 medium into a culture vessel and equilibrate to 37°C and 5% CO_2.
2. Passage CHO cells from serum supplemented culture into BalanCD CHO DG44 at $3x10^5$ viable cells/mL. It is important that cells be in the logarithmic phase of growth with at least 90% viability before passaging.
3. Incubate cultures at 37ºC and 5% CO_2 until the viable cell density reaches $1x10^6$ viable cells/mL.
4. Subculture into fresh BalanCD CHO DG44 medium at $3x10^5$ viable cells/mL starting density.
5. Maintain cells in BalanCD CHO DG44 for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

II. Sequential Adaptation from Serum-Supplemented Media to BalanCD CHO DG44

Sequential adaptation may be used if direct adaptation is troublesome.

1. Dispense the original serum-supplemented medium and BalanCD CHO DG44 medium in a 3:1 ratio into an appropriate culture vessel and equilibrate to 37°C and 5% CO_2.
2. Passage CHO cells from serum-supplemented culture into 3:1 BalanCD CHO DG44 at $3x10^5$ viable cells/mL. It is important that cells be in the logarithmic phase of growth with at least 90% viability before passaging.
3. Incubate cultures at 37ºC and 5% CO_2 until the viable cell density reaches $1x10^6$ viable cells/mL.
4. Subculture at $3x10^5$ viable cells/mL starting density into fresh medium prepared in a 2:1 ratio of original serum-supplemented medium to BalanCD CHO DG44.
5. Repeat steps 3 and 4 with sequential dilution ratios of 1:1, 1:2, 1:4, and 0:1 of the original serum-supplemented medium and BalanCD CHO DG44. If the cells look unhealthy or the growth rate declines significantly at a particular step of adaptation, maintain the cells for an additional passage in the media ratio of the previous step before subculturing into the next ratio.
6. Maintain cells in BalanCD CHO DG44 for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

Sub-culturing Procedure

Suspension culture conditions:
• Culture vessels: Shake Flasks (Corning #431143-431147 or equivalents)
• Working volume: 30 mL for 125 mL flask
• Seeding density: 2-4 x 10^5 cells/mL
• Incubator: 37 °C, 5% CO_2, humidified, 120 rpm

1. Dispense BalanCD CHO DG44 medium into a culture vessel and equilibrate to 37 ºC and 5% CO_2.
2. Passage cells into BalanCD CHO DG44 medium at 5 x 10^5 viable cells/mL. It is important that cells be in the logarithmic phase of growth with at least 90% viability before passaging.
3. Incubate cultures for 3-4 days or until the viable cell density reaches 3-5 x 10^6 viable cells/mL.
4. Maintain cells in BalanCD CHO DG44 for several passages, sub-culturing twice weekly to allow complete adaptation and assure optimum performance. A minimum of three passages in BalanCD CHO DG44 is strongly recommended before use.

Cryopreservation

FREEZING

1. Use cultures that are in logarithmic growth with >90% viability.
2. Prepare required volume of freezing medium - 90% cold BalanCD CHO DG44 + 10% DMSO (Sigma Aldrich D2650 or equivalent). Keep at 4 °C until use.
3. Centrifuge cells for 5 minutes at 200 *x g*.
4. Re-suspend cells in cold freezing medium to reach 1 x 10^7 viable cells/mL or desired cell density.
5. Aliquot 1 mL or desired volume into sterile cryovials.
6. Gradually lower the temperature of the vials to -80 °C at a rate of -1 °C/minute.
7. Store vials in liquid nitrogen vapor phase.

THAWING

1. Thaw frozen vial rapidly in a 37 °C water bath.
2. Transfer the cells to a centrifuge tube containing 10 mL of pre-warmed BalanCD CHO DG44 medium.
3. Centrifuge for 5 minutes at 200 *x g* and decant the supernatant without disturbing the cell pellet.
4. Re-suspend the cell pellet in the medium and transfer the cell suspension to a culture flask with pre-warmed BalanCD CHO DG44 medium to achieve an initial cell density of 3x10^5 cells/mL. Incubate cultures at 37 °C and 5% CO_2 for 3-4 days.
5. Sub-culture cells following the sub-culture procedure. Cells usually grow slowly in the first 1-2 passages. A minimum of three passages in BalanCD CHO DG44 is strongly recommended before use.

Fed-Batch Applications

BalanCD CHO DG44 medium was designed to support growth and productivity of dihydrofolate reductase deficient (DHFR) Chinese Hamster Ovary (CHO) cells in a fed-batch process with BalanCD CHO Feed 4 (Cat # 94134). Cell lines vary, thus customers are encouraged to determine an optimal volume and feed schedule for their specific cell line. BalanCD CHO DG44 medium was tested across multiple cell lines that required distinct feeding schedules. Please see Table 1 below or visit http://www.irvinesci.com/products/94134-balancd-cho-feed-4-powder for a Feed Method Optimization Guideline located in the BalanCD CHO Feed 4 product insert.

Briefly, BalanCD CHO Feed 4 should be prepared at the desired concentration and an optimal total feed volume between 10-30% of the initial culture volume should be determined. In a subsequent experiment, once a feeding volume has been determined, a feeding schedule should be optimized as depicted in the table below, where a feed volume of 20% is distributed over the length of the fed-batch process.

Table 1: Example Feed Schedule optimization using 20% feed volume.

Feed Schedule										
Culture Day	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
FB-1	4%	4%	4%	4%	4%					
FB-2		4%	4%	4%	4%	4%				
FB-3			4%	4%	4%	4%	4%			
FB-4				4%	4%	4%	4%	4%		
FB-5					4%	4%	4%	4%	4%	
FB-6	4%		4%		4%		4%		4%	
FB-7		4%		4%		4%		4%		4%
FB-8			4%		4%		4%		4%	
FB-9	3%	3%	3%	3%	3%					
FB-10	5%	5%	5%	5%	5%					

Example Data: Optimizing Feed for Productivity

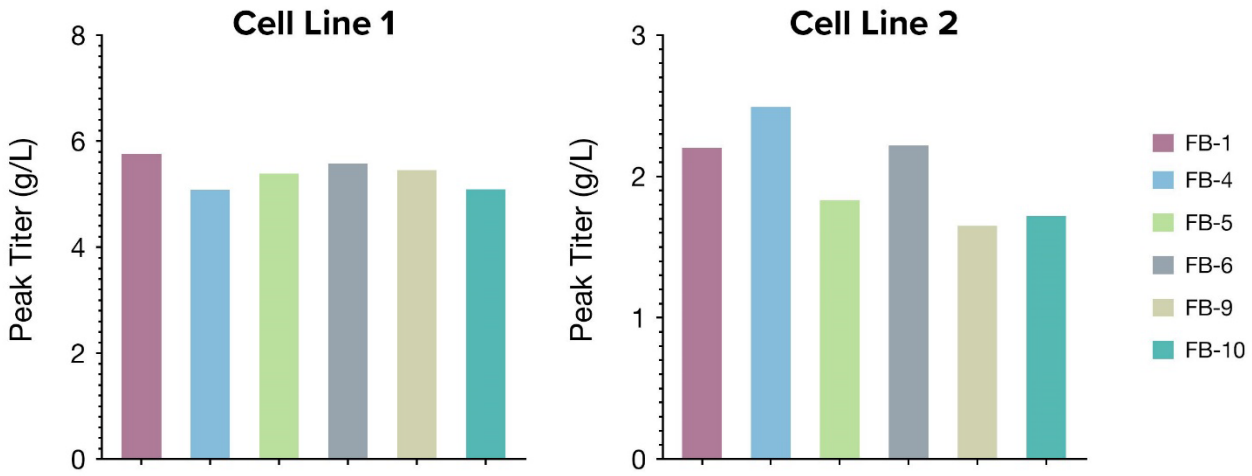

Figure 1. Peak titers of multiple DG44 cell lines with BalanCD CHO DG44 growth medium fed with BalanCD CHO Feed 4 using different feeding schedules and volumes. Culture vessels were sampled at regular intervals during a 14-day fed-batch process and measured for titer using bilayer interferometry. Cell productivity varied between cell lines and the feeding strategy, demonstrating the need to optimize the feeding strategy for each cell line, as Cell Line 1 did not show significant differences, while Cell Line 2 benefited from daily feeding from day 6 to 10 compared to other strategies with regard to peak titers.

Related Products

Catalog #	Product	Available Package Sizes*
94134	BalanCD CHO Feed 4	1 L, 10 L
9317	L-Glutamine Solution (200 mM)	100 mL, 500 mL
9309	Water for Injection (WFI)	1L, 20L, 200 L
9240	1X PBS, Dulbecco's Phosphate Buffered Saline	100 mL, 500 mL, 1 L

* Additional package sizes are available upon request

Technical Support

CONTACT US

For more information or assistance, contact Customer Service at:

- Email: fisitmrequest@fujifilm.com
- Direct line: US +1 800 577 6097
  EU +31 13 5791911

WEBSITE RESOURCES

Visit the website at www.irvinesci.com for technical resources and information including:

- Safety Data Sheets (SDS)
- COAs (when available)
- FAQs (when available)
- Product literature
- Complete list of offices and contact information by country

FUJIFILM Irvine Scientific, Inc.
2511 Daimler Street, Santa Ana, California 92705 USA
Telephone: 1 949 261 7800 • 1 800 437 5706
Fax: 1 949 261 6522 • www.irvinesci.com

P/N 014436, Rev.00
Effective Date: