BalanCD CHO Perfusion

Catalog #	Product	Format	Available Package Sizes*
91178	BalanCD CHO Perfusion	Liquid	1 L
94149	BalanCD CHO Perfusion	Powder	10 L

* Additional package sizes are available upon request

Intended Use

For further manufacturing use only.

Product Description

BalanCD CHO Perfusion is a chemically defined and animal component-free complete growth medium for Chinese Hamster Ovary (CHO) cells specifically designed for growth and productivity in a continuous steady-state perfusion process. BalanCD CHO Perfusion is provided without L-Glutamine to extend shelf life. The recommended L-Glutamine concentration to add is 4-8 mM (20-40 mL/L). Glucose should be monitored during culture as additional supplementation may be required. BalanCD CHO Perfusion has been designed to support high cell densities of CHO cell lines in perfusion-capable bioreactors at a minimum of 1 vessel volume per day. The medium does not contain antibiotics, antimycotics, protein hydrolysates or any other undefined components.

Shipping

Product is shipped at ambient temperature. Upon receipt, store immediately at 2-8 °C, protected from light.

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, un-opened powder medium (Cat #94149) is stable for 24 months from date of manufacture. 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, un-opened liquid medium (Cat #91178) is stable for 12 months from date of manufacture. Do not use after the assigned expiration date.

Directions for Use

Hydration from Powder Medium

1. Add powder medium Catalog #94149 (29.65 g/L) to 90% of the 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 and clear.
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. Add additional water to bring solution to final desired volume.
6. Measure osmolality. Final osmolality is expected to be 285-310 mOsm/kg.
7. Sterile filter through a 0.2 µm filter membrane.
8. Store at 2-8 °C, in the dark for up to 1 year. Avoid prolonged exposure at room 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.

Adaptation

I. Direct Adaptation to BalanCD CHO Perfusion

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

1. Dispense BalanCD CHO Perfusion 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 Perfusion 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 Perfusion medium at $3x10^5$ viable cells/mL starting density.
5. Maintain cells in BalanCD CHO Perfusion for several passages, subculturing twice weekly to allow complete adaptation.

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

Sequential adaptation may be used if direct adaptation is troublesome.

1. Dispense the original serum-supplemented medium and BalanCD CHO Perfusion 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 Perfusion 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 Perfusion.
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 Perfusion. 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 Perfusion for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

Subculturing Procedure

Suspension culture conditions:

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

1. Dispense BalanCD CHO Perfusion medium into a culture vessel and equilibrate to 37 ºC and 5% CO_2.
2. Passage cells into BalanCD CHO Perfusion 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 Perfusion for several passages, sub-culturing twice weekly to allow complete adaptation and assure optimum performance. A minimum of three passages in BalanCD CHO Perfusion 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 Perfusion + 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 Perfusion 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 Perfusion 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 Perfusion is strongly recommended before use.

Perfusion Applications

BalanCD CHO Perfusion medium can support a range of continuous or steady-state perfusion culture applications, including small-scale perfusion-mimic systems and perfusion-capable bioreactors. The medium is designed to support cell growth and productivity at 1 vessel volume per day (VVD). A predetermined steady state VCD should be used based on either the knowledge of the cell line or determined experimentally. When utilizing a bioreactor, instrument parameters should be optimized for best results with the BalanCD CHO Perfusion Medium.

Determine Steady State VCD Experimentally

Prior to initiating a bioreactor perfusion experiment, a spin tube (Corning Mini Bioreactor Centrifuge Tube, Fisher Scientific Cat # 07202150) or a microbioreactor (i.e. Ambr®15) can be used to determine the target steady-state of the cell line. The suggested protocol below utilizes a spin tube perfusion-mimic model to determine the maximum VCD that BalanCD CHO Perfusion can attain.

1. Using cells in the logarithmic phase of growth, seed cells at a VCD of 2 x 10^6 cells/mL into a spin tube containing 10 mL of evaluation medium, incubate cells at 37°C, 5% CO_2, with agitation specific for your cell line.
2. Perform a Day 0 count after seeding to confirm seeding density.
3. After 24 hours, perform a cell count and begin media exchanges daily for up to 14 days.
4. After counting the cells, perform a media exchange by centrifuging the spin tube at 200 – 250 x g for 5 minutes.
5. For one vessel volume per day (VVD), remove entire supernatant volume, leaving the cell pellet undisturbed. Note: The culture vessel will contain residual medium.

6. For each vessel, using a serological pipette, add 10 mL of fresh media and pipette gently up and down to resuspend cells. (Alternatively, disrupt the pellet prior to adding fresh media by gently flicking the vessel to minimize cell loss. Then, add the fresh medium.)
7. Once media exchange is complete, return the vessels to the incubator.
8. Media removed from the culture can be used to measure metabolites (pH, Gln, Glu, Glucose, Lactate, Ammonium), osmolality, productivity, and/or to evaluate product quality.
9. Continue steps 3 through 8 above for up to 14 days or until cells drop in viability below 80%.
10. Determine the steady state VCD based on your preferred metrics from the data, including but not limited to growth and titer. Product quality attributes (PQA) can also be considered.

Related Products

Catalog #	Product	Available Package Sizes*
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

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