BalanCD® Transfectory™ CHO

Product Manual

BalanCD® Transfectory™ CHO is a chemically-defined, animal derived component-free medium optimized for both transient transfection and production of CHO cells. The medium contains no hydrolysates or any other undefined components. This medium is supplied ready to use for suspension culture applications.

BalanCD Transfectory CHO is formulated without L-Glutamine, antibiotics, or antimycotics

Catalog #	Product	Size
91147	BalanCD Transfectory CHO	1L Liquid Standard
94129	BalanCD Transfectory CHO	Dry Powder Format, 10L, 100L
91148	Transfectory Supplement	500mL Liquid Standard
91150	Anti-Clumping Supplement	50mL Liquid Standard

Intended Use

For research or further manufacturing use only.

Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis which is available upon request.

Storage Instructions and Precautions

Liquid Medium

Handle using aseptic techniques to avoid contamination. Store at 2-8°C away from light. Do not use after the assigned expiration date. Do not use any bottle of medium that shows evidence of particulate matter or cloudiness.

Powder Medium

Store at 2-8°C protected from atmospheric moisture. This product is very hygroscopic. Bring it to room temperature before opening and make sure to re-seal tightly after opening. The powder should be free flowing; do not use if it is caked. Do not use after the assigned expiration date.

Media Preparation

Reconstitution from powder medium

1. Measure 1000 mL/L WFI (Catalog # 9309 or equivalent) into appropriate sized container.
2. Add 23.41 g/L of BalanCD Transfectory CHO powder (Catalog # 94129) to water. Mix for approximately 10 minutes. Solution will be slightly cloudy.
3. Add 2.2 g/L Sodium Bicarbonate to solution. Stir for an additional 10 minutes or until solution is clear.
4. Measure pH (expected range 7.0-7.4) and osmolality (expected range 280-310mOsm/kg).
5. Sterile filter through a 0.2 µm filter.
6. Store at 2-8°C for up to 1 year (protect from light).
   Note: Do not use if solution has precipitated or changed color.
7. Supplement with 20 mL/L of 200 mM L-Glutamine (Catalog # 9317) to reach 4 mM final concentration prior to use.

Media Supplements

- Supplement with 20 mL/L of 200 mM L-Glutamine (Catalog # 9317) to reach 4mM final concentration prior to use.
- Optional Supplements
  - 2mL/L Anti-Clumping Supplement (Catalog # 91150): Anti-Clumping Supplement is intended for cell stock maintenance and post transfection cultures only. Cells must be spun down and re-suspended in media without Anti-Clumping Supplement before transfection.
    Note: Anti-Clumping Supplement will completely inhibit transfection
  - Poloxamers (Pluronics® or Kolliphor®): BalanCD Transfectory CHO medium contains poloxamers but additional 0.05%-0.1% can be supplemented to minimize sheer stress in the cultures.
  - Transfectory Supplement (Catalog # 91148): Supplement 10% of the initial culture volume one day *after* transfection to boost cell growth and titer. This supplement is animal-component-free.

Culture Conditions

- Medium: BalanCD Transfectory CHO supplemented with 4mM L- Glutamine (Catalog # 9317)
- Culture vessels: Vented Baffled Polycarbonate Shake Flasks (Corning # 431405-431407 or equivalents)
- Culture volume: 30-40mL in 125mL flasks, 60-80mL in 250mL flasks
- Seeding density: 2-3 x10^5 cells/mL
- Incubator setting: 37°C, 5% CO_2, humidified, 140 rpm

Cell Recovery and Adaptation

1. Supplement BalanCD Transfectory CHO medium (Catalog # 91147) with 4mM of L-Glutamine (Catalog # 9317) and 2mL/L of Anti-Clumping Supplement (Catalog # 91150). Aseptically transfer appropriate volume (30mL) of supplemented media into a 125mL shake flask and pre-warm in the incubator.
2. Thaw frozen vial rapidly in a 37°C water bath.
3. Transfer the cells to a centrifuge tube containing 10mL of cold BalanCD Transfectory CHO medium.
4. Centrifuge for 5 minutes at 200g and decant the supernatant without disturbing the cell pellet.
5. Re-suspend the cell pellet in the medium and transfer the cell suspension to a culture flask with pre-warmed BalanCD Transfectory CHO medium (step 1) to achieve an initial cell density of 3 x 10^5 cells/mL. Incubate cultures for 3-4 days.
6. Cells can be directly adapted into BalanCD Transfectory CHO medium. After a minimum of three passages in BalanCD Transfectory CHO, viable cell density and percent viability should reach above 1.5x10^6 cells/mL and 90% within 4 days, if they are successfully adapted.
7. If severe cell aggregation is observed, increase amount of Anti-Clumping Supplement to 2-4mL/L.
8. If cells grow slowly (less than 1.5x10^6cells/mL within 4days) with viability below 90%, continue passaging with the following recommendations.
    - Increase seeding density to 0.5-1x10^6 cells/mL.
    - Spin down and re-suspend cells into fresh medium at each passage.
    - Sequential adaption at ratios of 1:1, 1:2, 1:4, and 0:1 of the original medium and BalanCD Transfectory CHO medium

Subculture

1. Add 4mM of L-Glutamine (Catalog # 9317) to BalanCD Transfectory CHO medium (Catalog # 91147).
2. Subculture cell stocks every two to four days to keep cells in early logarithmic growth phase with a seeding density of 2-3 $x10^5$ cells/mL. Viable cell density and percent viability should reach above $2x10^6$ cells/mL and 90% within 4 days.
   Note: It is strongly recommended to keep the cultures under $3x10^6$cells/mL to achieve highest transfection efficiencies
3. Anti-Clumping Supplement may not be necessary at low cell densities (<$3x10^6$ cells/mL), but if cell aggregation is observed, supplement 2mL/L of Anti-Clumping Supplement.
   - **Note: Recommended at a concentration between 500x and 1000x (1mL/L – 5mL/L) depending on degree of clumping. Anti-Clumping Supplement will completely inhibit transfection. Cells must be spun down and re-suspended in media without Anti-Clumping Supplement before transfection.**

Cryopreservation

1. Prepare required volume of freezing medium: 90% cold BalanCD Transfectory CHO + 10% DMSO. Keep in 4°C until use.
2. Centrifuge appropriate quantity of healthy cells for 5 minutes at 200g and decant the supernatant without disturbing the cell pellet.
3. Re-suspend cells in cold freezing medium to reach $1x10^7$ viable cells/mL or desired cell density.
4. Aliquot 1mL or desired volume into sterile cryovials.
5. Gradually lower the temperature of the vials to -80°C at a rate of -1°C/minute.
6. Store vials in liquid nitrogen vapor phase.

Transient Transfection – Example Method and Data

The following PEI-mediated transient transfection protocol can be used as a general guideline to begin optimization work. Since optimal transfection parameters may vary from different culture applications, optimization of transfection parameters is highly encouraged.

BalanCD Transfectory CHO medium is also compatible with other transfection methods using cationic liposomes or electroporation.

Culture condition used in this example

- Cells: CHO-3E7 cells (Canadian National Research Council L-11992)
- Medium: BalanCD Transfectory CHO + 4 mM L-Glutamine
- Culture vessels: Corning 125mL Erlenmeyer shake flask (Corning #431143 or equivalent)
- Working Volume: 30mL
- Seeding density: 2 x10^5 cells/mL
- Rotation speed: 140 rpm
- Incubator: 37°C, 5% CO_2, humidified
- Cell Counts: Beckman Coulter, ViCell® XR Cell Viability Analyzer
- Protein Concentration: Octet QKe system (fortéBIO)
- PEI Transfection
    - Transfection VCD at 3x10^6 cells/mL
    - DNA to PEI ratio at 1:5
        - ✓ DNA: 1µg/mL in final culture volume, 40:40:15:5 Hc:Lc:Akt:GFP
        - ✓ PEI (PEIpro, PolyPlus #115-010 or equivalent): 5µg/mL in final culture volume

    Note: It is recommended the amount of DNA and PEI be optimized for each application
- One day post-transfection
    - Temperature shift to 32°C
    - Transfectory Supplement: 10% working volume
    - Anti-Clumping Supplement: 2mL/L

Procedure

1. Measure VCD of cell stock and calculate required volume.
2. Centrifuge cells for 5 minutes at 200g and decant the supernatant without disturbing the cell pellet.
3. Re-suspend the cell pellet in the medium and transfer the cell suspension to a culture flask with 27mL of BalanCD Transfectory CHO medium (with 4mM L-Glutamine) to achieve an initial cell density of 2 x 10^5 cells/mL.
4. Incubate cultures for 3 days. VCD should be 2.5 - 3x10^6 cells/mL.

   Note: Cells should be in the early logarithmic growth phase for optimal transfection and transfection density should be optimized for each culture application.

5. In a separate tube, prepare 3mL of Polyplex (10µg/mL DNA and 50µg/mL PEI) – add appropriate volume of DNA and polyethylenimine stocks in BalanCD Transfectory CHO medium.
6. Incubate the mixture at room temperature for *7 minutes* and add the entire amount (3mL) to the flask containing 27mL of culture.
7. One day post-transfection, adjust the temperature to 32°C and add 60uL of Anti-Clumping Supplement (2mL/L, Catalog # 91150) to the flask containing 30mL of culture.
8. To achieve higher cell growth and titer, add 3mL of Transfectory Supplement (10% of culture volume, Catalog # 91148) one day after transfection.
9. Monitor and maintain glucose level at a 2-8g/L range throughout the culture.

Example Data

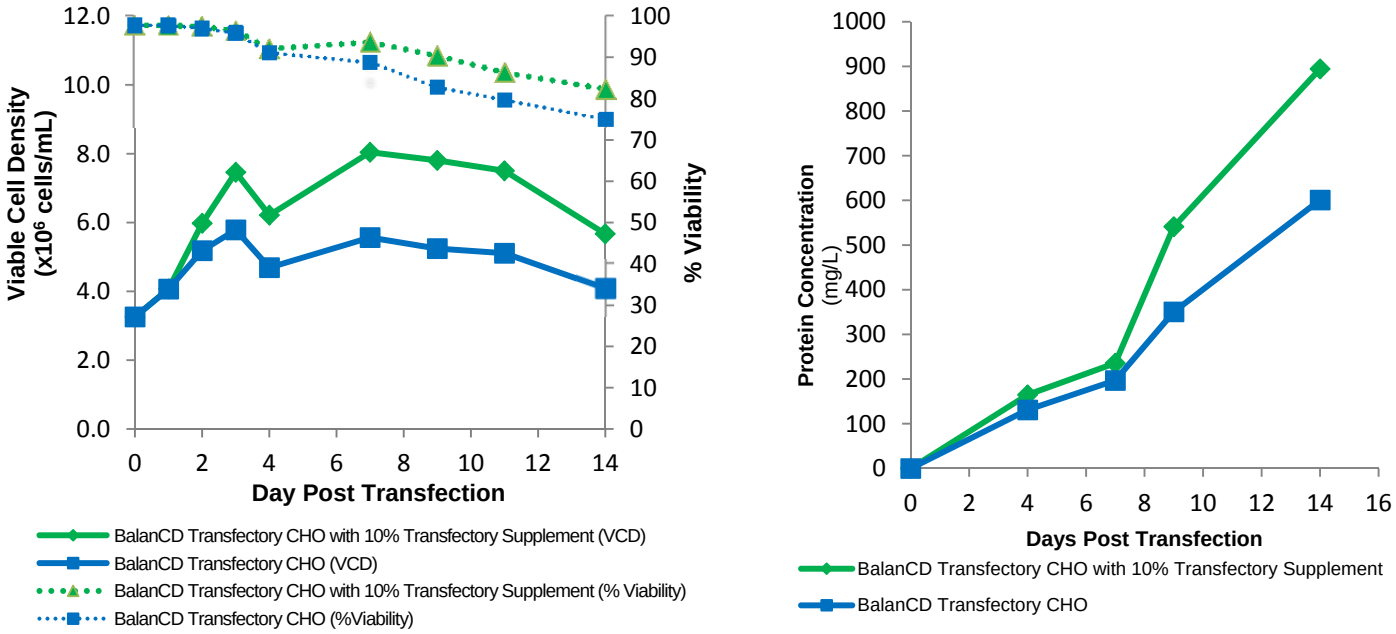

Figure 1. PEI-mediated transient transfection in shake flasks - cell growth and protein production.

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