SAFC®
Pharma/Biopharma Raw Materials

Technical Bulletin

# CHOZN® CHO K1

## Catalog NO. CHOK1
## (CHOZN® CHO K1 Host Cell Line)

### Product Description/Overview

The CHOZN® CHO K1 line is a suspension CHO K1 cell line, adapted for growth in chemically defined media. The cell line comes with full traceability documentation, and was banked in cGMP conditions. Several cGMP produced chemically defined media are recommended for use with the CHOZN® CHO K1 cell line. The media and feed formulations were developed using animal component free raw materials that have a proven track record for sourcing and robust manufacturability.

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MilliporeSigma

## Precautions and Disclaimer

The CHOZN® CHO K1 cell line and associated media and feed are for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheets for information regarding hazards and safe handling practices.

## Storage and Stability

Immediately upon receipt, store the cells in the vapor phase (approx –150 °C to –180 °C) of liquid nitrogen.

## Media, Feeds, and Supplements used with the CHOZN® CHO K1 Cell Line

*Note: please refer to the Product Information Sheet/ Technical bulletin for each of these products for information on reconstitution techniques, storage and stability.*

### Growth media: EX-CELL® CD-CHO Fusion Medium without L-glutamine (14365C-liquid/24365C-dry powder) (cGMP)

EX-CELL® CD CHO Fusion Medium is a chemically defined, animal-component free medium developed for the long-term growth of CHO cells. The absence of any large macromolecules allows for streamlined isolation and purification of secreted proteins from the cells. This medium does not contain L-glutamine, which improves medium stability, eliminates L-glutamine degradation that causes ammonia build-up, and provides an appropriate medium for the culture of CHO cells that use GS selection. EX-CELL® CD CHO Fusion Medium is manufactured under cGMP production quality conditions with full raw material documentation and is available in liquid or powder format.

### Additional recommended but not required media and feeds Cloning media: EX-CELL® CHO Cloning Medium without L-glutamine (C6366) (cGMP)

EX-CELL® CHO Cloning medium is an animal-component free medium designed to support clonal survival and growth of Chinese Hamster Ovary (CHO) cell lines, with results comparable to the traditional method using 10% fetal bovine serum. Developed to meet the needs of the biotechnology industry, this medium is designed for single-cell cloning of recombinant CHO cell lines adapted to serum-free suspension culture. This medium does not contain L-glutamine, which improves medium stability, eliminates L-glutamine degradation that causes ammonia build-up, and provides an appropriate medium for the culture of CHO cells that use GS selection. This medium is manufactured under cGMP production quality conditions with full raw material documentation and is available in a liquid format.

### Fed batch medium: EX-CELL® Advanced™ CHO Fed Batch medium (14366C-liquid/ 24366C-dry powder)

EX-CELL® Advanced™ CHO Fed-Batch Medium is a chemically defined, next generation media platform. The formulation was developed using multivariate analysis of 10,000+ data points that included performance, physical, regulatory and safety design specifications. This medium is designed to be used in conjunction with Advanced™ CHO Feed 1 for superior platform performance in fed-batch cultures.

### Feed: EX-CELL® Advanced™ Feed 1 (24367C w/ glucose /24368C w/out glucose)

EX-CELL® Advanced™ CHO Feed 1 is a single part, next generation feed with highly concentrated key critical raw materials. The formulation was developed using multivariate analysis of 10,000+ data points that included performance, physical, regulatory and safety design specifications. This feed is designed to be used in conjunction with Advanced™ CHO Fed-batch Medium for superior titer performance in fed-batch cultures.

## Required Cell Culture Reagents

| Cell Culture Reagents | Manufacturer | Cat. No. |
|---|---|---|
| CHOZN® CHO K1 cells | SAFC | **CHOK1** |
| EX-CELL® CD CHO Fusion Medium | SAFC | **14365C (liquid)<br>24365C (powder)** |
| L-glutamine (200 mM) | Sigma-Aldrich SAFC | **G7513 59202C** |
| Dimethyl sulfoxide (DMSO) | Sigma-Aldrich | **D2438** |
| Sodium Bicarbonate | Sigma-Aldrich | **S5761** |
| 36–38% Hydrochloric Acid | Sigma-Aldrich | **H1758** |
| 50% Sodium Hydroxide | Sigma-Aldrich | **415413** |
| Tissue Grade Water | Sigma-Aldrich | **W3500** |

### Recommended but not Required Cell Culture Reagents

| Cell Culture Reagents | Manufacturer | Cat. No. |
|---|---|---|
| EX-CELL® CHO Cloning Medium | SAFC | **C6366** |
| EX-CELL® Advanced™ CHO Fed-batch Medium | SAFC | **14366C (liquid) 24366C (dry powder)** |
| EX-CELL® Advanced™ Feed 1 | SAFC | **24367C (w/ glucose) 24368C (w/out glucose)** |
| D-(+)-Glucose (45% solution) | Sigma-Aldrich | **G8769** |

## Equipment/materials/reagents needed

- Expression vector (plasmid DNA) containing gene expression cassettes for the protein of interest and appropriate selection cassette (example: Puromycin) (recommended plasmid concentration ~1µg/µl)
- Sterile filtration unit; 0.22 µm, 1000 mL capacity (Millipore® Stericup™ SCGPU10RE or equivalent)
- Low-volume pipettes and sterile tips (Rainin® Classic set or equivalent)
- Sterile pipettes (1 mL, 2 mL, 5 mL, 10 mL, 25 mL, 50 mL)
- T-25 $cm^2$ and T-75 $cm^2$ suspension cell (hydrophobic surface treated) culture flasks (Greiner Bio-one 690195 and 658195 or equivalent)
- 15 mL and 50 mL sterile conical centrifuge tubes (Corning 430052 and 430290 or equivalent)
- 125 mL sterile Erlenmeyer non-baffled, vented cap culture flask (Corning 431143 or equivalent)
- 50 mL TPP (Techno Plastic Products) TubeSpin® tubes
- Sterile microfuge tubes (1.5 mL Eppendorf® or equivalent)
- 4 mm Electroporation Cuvettes (Sigma Z706094 or equivalent)
- Electroporator: Bio-Rad Laboratories, Inc. (Genepulser® or similar electroporation instrument)
- Refrigerated centrifuge with swinging bucket rotor (capable of 3000x g forces)
- $CO_2$ incubator (5% $CO_2$, 37 °C, humidified)
- Automated cell counter or hemocytometer
- Orbital shaker plate or $CO_2$ shaker culture incubator system (ATR Multitron II or similar)

- Water bath set at 37 °C
- Biological safety cabinet (Class II; Type A2; ISO 5)
- Refrigerator (4 °C)
- Freezer (–20 °C)
- Ultra-cold freezer (–80 °C)
- Liquid nitrogen ($LN_2$) freezer; recommend vapor phase (–150 °C to –196 °C)
- Sterile cryovials (Nalgene 5000–1020 or equivalent)
- Cryovial labels ($LN_2$ resistant)
- $LN_2$ freezer boxes
- Controlled rate freezing vessel (Nalgene “Mr Frosty” or controlled rate freezer equipment capable of a cooling rate of approximately 1C/min.)
- 70% isopropanol (2-propanol; 70% in $H_2O$, Sigma 563935)

## CHOZN® CHO K1 User Protocols

**NOTE: The following procedures should only be performed by personnel trained to:**

- Work with potentially biohazardous materials
- Handle all cell culture procedures under at least Biosafety Level 1 (BSL-1) containment and practices
- Use Universal precautions for biosafety (WHO Laboratory Biosafety Manual; 3rd ed., 2004)
- Use aseptic technique for all cell and media handling procedures

***Note: All cell culture and media handling in these protocols must be carried out in a HEPA filtered (Class II) biological safety cabinet capable of creating an ISO Class 5 clean environment.***

## Part I: Media Preparation for Initial Thaw and Bank of CHOZN® CHO K1 Cells

***Note 1: Please refer to the product guides for recommendations on reconstitution, storage and stability information on all other media and feeds.***

### EX-CELL® CD CHO Fusion Liquid Medium Supplemented with 4 mM L-glutamine (referred to throughout the technical bulletin as "Growth Medium")

### Reagents and Equipment

- EX-CELL® CD CHO Fusion medium (Sigma 14365C)
- Sterile filtration unit 0.22 µm (1000 mL capacity)
- Sterile pipettes
- Biological safety cabinet (Class II; Type A2; ISO 5)
- Freezer (–20 °C)
- L-glutamine; 200 mM (Sigma G7513)
- Refrigerator (2–8 °C)
- Water bath set at 37 °C
- 70% isopropanol (in spray bottle for surface decontamination)

### Procedure

The following procedure describes the preparation of 1L of Growth Medium (EX-CELL® CD CHO Fusion supplemented with 4 mM L-glutamine):

i) Prepare supplements

   a. L-glutamine: Thaw 200 mM stock bottle of L-glutamine in a 37 °C water bath until completely dissolved. After thawing, store any unused L-glutamine at 2–8 °C for up to two weeks.

ii) Aseptically add 20 mL of 200 mM L-glutamine into 1L of EX-CELL® CD CHO Fusion according to the chart below.

iii) (Optional) Filter the complete medium through a 1000 mL capacity sterile filtration unit to ensure sterility of the medium.

iv) Mark the date of preparation on the medium bottle and store medium at 2–8 °C in the dark until needed.

v) Discard any unused glutamine-supplemented medium after one month.

**Growth Medium (EXCELL® CD CHO Fusion + 4 mM glutamine) for CHOZN® CHO K1 Parental Cells**

| Material | Product Number (Sigma-Aldrich) | Volume Needed | Final Concentration |
|---|---|---|---|
| EX-CELL® CD CHO Fusion | **14365C** | 1L | 1X |
| L-glutamine (200 mM) | **G7513** | 20 mL | 4 mM |

## Part II: Stock Culture Initiation/Thawing and Sub-culturing of CHOZN® CHO K1 Cells (cGMP banked).

### Purpose

This protocol describes procedures for the stock initiation of CHOZN® CHO K1 cells.

### Reagents and Equipment

- T-75cm$^2$ suspension cell (hydrophobic surface treated) culture flasks (Greiner Bio-one 690195 or equivalent)
- 15 mL sterile conical centrifuge tube (Corning 430052 or equivalent)
- 125 mL sterile, non-baffled, vented cap Erlenmeyer culture flask (Corning 431143 or equivalent)
- 50 mL TPP (Techno Plastic Products) TubeSpin® tubes
- Sterile pipettes
- Frozen vial of CHOZN® CHO K1 cells (Sigma CHOK1)

***Note: Cells are banked at approximately 10e6 cells/ml in 93% Growth Medium (EX-CELL® CD-CHO Fusion with 4 mM L-glutamine) and 7% Dimethyl sulfoxide (DMSO).***

- EX-CELL® CD CHO Fusion cell culture Growth Medium (prepared as described in Part I containing 4 mM L-glutamine)
- Water bath at 37 °C
- Biological safety cabinet (Class II; Type A2; ISO 5)
- Centrifuge
- $CO_2$ incubator (5% $CO_2$, 37 °C,humidified)
- Automated cell counter or hemocytometer
- Orbital shaker plate (set at 125 rpm for Erlenmeyer flasks, 200 rpm if TPP® TubeSpin tube)
- 70% isopropanol in a spray bottle

### Procedure

i) Thawing the cells

a. Adjust incubator settings to 37 °C and 5% $CO_2$. Humidify the unit by placing a shallow pan of sterile water near bottom of incubator or set to 80% if humidity control feature is available.

b. Pre-warm growth medium to 37 °C in a water bath.

***Note: Do not allow the medium to be in the water bath for greater than 1–2 hours.***

c. Obtain one sterile T-75 cm$^2$ suspension cell (hydrophobic surface treated) culture flask.

d. Obtain a sterile 15 mL sterile conical centrifuge tube.

e. Aseptically transfer 8 mL of pre-warmed, sterile EX-CELL® CD CHO Fusion cell culture Growth Medium (with 4 mM L-glutamine) into the 15 mL sterile conical centrifuge tube.

f. Obtain a frozen vial of the CHOZN® CHO K1 cells (1 mL) from the $LN_2$ freezer.

g. Immediately thaw the vial by gently swirling the vial in a 37 °C water bath until just thawed (approximately 1 minute). Do not completely submerge the vial to avoid contamination.

h. Remove the vial from the water bath and spray the vial with a copious amount of 70% isopropyl alcohol to decontaminate the outside surfaces. Place vial in the biological safety cabinet.

i. Allow alcohol to completely dry from surface of vial before opening.

ii) Wash the cells

a. Aseptically transfer cells from the cryovial into the sterile 15ml conical centrifuge tube containing fresh EX-CELL® CD CHO Fusion cell culture Growth Medium (with 4 mM L-glutamine.

b. Centrifuge the cell suspension at 220 rcf for 5 minutes at 15–20 °C to pellet the cells.

c. Using a sterile pipet, carefully aspirate off the clarified medium taking care not to disturb the cell pellet. Discard the aspirate while retaining the cell pellet.

***Note: Caution- Clarified medium contains DMSO. Dispose of properly according to local regulations.***

iii) Sub-culturing the cells

a. Aseptically add 10 mL of fresh EX-CELL® CD CHO Fusion cell culture Growth Medium (with 4 mM L-glutamine) to the conical centrifuge tube containing the cell pellet. Gently re-suspend the cell pellet by pipetting up and down to break up the cell clumps.

b. Aseptically transfer the entire cell suspension (10 mL) to the sterile T-75 $cm^2$ suspension cell (hydrophobic surface treated) culture flask.

c. Incubate the cells in a 37 °C, humidified $CO_2$ incubator (non-shaking) for 20–28 hours.

***Note: The majority of the cells will not adhere to the flask. Use caution when transporting.***

d. After 24 hours, determine and record cell density and viability.

***Note: If stored under optimal storage conditions (vapor phase of liquid nitrogen, cells should recover within 24 hours and be >90% viable.***

e. Passage the cells by transferring the 10 mL of static culture from the T-75 $cm^2$ flask into a 125 mL sterile Erlenmeyer culture shaker flask (vented cap, non-baffled) containing an additional 10 mL of fresh Growth Medium (total volume approximately 20 mL). Place the flask on the orbital shaker plate, and shake at 125–130 rpm.

***Note: If using TPP tubes in place of Erlenmeyer flasks, adjust shaker speed setting to 200rpm.***

f. Once in shaker flask culture, maintain the cell culture stock by following the stock maintenance protocol described in Part III.

## Part III: Stock Maintenance of CHOZN® CHO K1 Cells

### Purpose

This protocol describes procedures for stock maintenance of the parental CHOZN® CHO K1 cells.

### Reagents and Equipment

- 125 mL sterile Erlenmeyer culture flask (non-baffled, vented cap) Corning 431143 or equivalent
- 50 mL TPP (Techno Plastic Products) TubeSpin® tubes
- Growth Medium (EX-CELL® CD CHO Fusion with Glutamine- prepared as described in Part I)
- Orbital shaker plate (set at 125–130 rpm in Erlenmeyer flasks; 200 rpm if TPP)
- Water bath (set to 37 °C)
- $CO_2$ incubator (5% $CO_2$, 37 °C, humidified)
- Sterile pipettes
- Biological safety cabinet (Class II; Type A2; ISO 5)
- Automated cell counter or hemocytometer
- 70% isopropanol in a spray bottle

### Procedure (to be performed every 3-4 days)

i) Verify that the incubator is set to 37 °C, 5% $CO_2$, and has water for humidity control (~80%).

ii) Pre-warm Growth Medium to room temperature or to 37 °C in a water bath.

iii) Aseptically remove a small volume of cell culture sample from the flask and count by trypan blue exclusion using a hemocytometer or an automated cell counter. Do not proceed if cell viability is less than 90%.

***Note: If cell viability is below 90%, troubleshoot conditions prior to continuing.***

iv) Determine the correct volume of cell culture to inoculate a new flask at a starting cell density of 0.3e6 cells/mL into the desired volume (see appropriate working volume for cell culture flasks in the table below).

v) Aseptically transfer the appropriate amount of cells to the new flask, and add pre-warmed growth medium up to the desired volume.

vi) Incubate flasks in a humidified 37 °C incubator with 5% $CO_2$ on an orbital shaker at 125–130 rpm.

***Note: If using TPP tubes in place of Erlenmeyer flasks, adjust shaker speed setting to 200 rpm.***

vii) Passage cells by repeating the above steps at least twice weekly, and expand culture volume as necessary according to the chart below.

### Appropriate Working Volume by Flask Size

| Shake Flask | Volume Range |
|---|---|
| 50 mL TPP® TubeSpin tube | 25–30 mL |
| 125 mL shaker flask | 17–35 mL |
| 250 mL shaker flask | 60–100 mL |
| 1L shaker flask | 300–400 mL |

## Part IV: Cryopreservation and Cell Banking of CHOZN® CHO K1 Cells

### Purpose

This protocol details procedures for establishing a working cell bank of CHOZN® CHO K1 cells.

### Reagents and Equipment

- Sterile cryovials (Nalgene 5000–1020 or equivalent)
- CHOZN® CHO K1 stock cell culture
- EX-CELL® CD CHO Fusion Growth Medium (prepared as in Part I)
- DMSO (Sigma D2438) Note: use fresh solution from an unopened bottle for best results
- Cryovial labels (must be $LN_2$ resistant)
- 15 mL and 50 mL sterile conical centrifuge tubes (Corning 430052 and 430290 or equivalent)
- Sterile pipettes
- Biological safety cabinet (Class II; Type A2; ISO 5)
- Centrifuge
- Controlled rate freezing vessel (Nalgene “Mr Frosty”) or controlled rate freezer equipment
- 70% isopropanol
- Ultra-cold freezer (–80 °C)
- $LN_2$ freezer boxes
- $LN_2$ freezer
- Automated cell counter or hemocytometer

### Procedure

i) If using a manual controlled rate freezing system, fill the vessel with fresh 70% isopropanol and/or follow manufacturer’s instructions.

ii) Label cryovials.

iii) Prepare Freezing Medium by aseptically adding fresh 100% DMSO to Growth Medium to achieve a final concentration of 7% DMSO.

iv) Aseptically remove a small volume of cell culture from the flask and count by trypan blue exclusion using a hemocytometer or an automated cell counter. Do not proceed if cell viability is less than 90%.

v) Calculate the volume of cell stock and Freezing Medium needed to obtain 5-10e6 cells per cryovial (1ml volume).

***Note: Once cell preparation is initiated, work must proceed quickly. It is recommended that the total time from removing cells from the stock culture to placing the controlled rate freezing vessel containing the banks into the –80 °C freezer take no more than 30 minutes.***

vi) Aseptically transfer calculated volume of stock culture to an appropriately sized sterile conical centrifuge tube.

vii) Centrifuge at 220 rcf for 5 minutes at 15–20 °C.

viii) Carefully aspirate off the supernatant taking care not to disturb the cell pellet.

ix) Gently re-suspend the cells with calculated volume of Freezing Medium.

x) Mix thoroughly by gently pipetting the cell suspension.

xi) Immediately aseptically aliquot 1.0 mL of the cell suspension into labeled cryovials. Cap tightly.

xii) Quickly transfer the vials to the prepared controlled rate freezer system vessels (manual) and transfer into a –80 °C freezer, or transfer vials into a controlled rate freezer and follow recommended procedures for overnight freezing.

xiii) Transfer frozen vials to the vapor phase of a $LN_2$ freezer within 18–72 hours of freezing.

## Part V: Transfection of CHOZN® CHO K1 Cells

### Purpose

This protocol describes procedures for transfection of the CHOZN® CHO K1 cells via electroporation. The transfection conditions provided in this protocol have been optimized for the CHOZN® CHO K1 parental cell line. When performed properly, a 60–80% transfection efficiency (determined by the expression of a fluorescent reporter construct in the transfected pool) should be achieved.

***Note: While not recommended, lipid based transfection methodologies may also be used but lower transfection efficiencies may result. The usage of transfection reagents that are designed for suspension CHO cells are recommended (i.e. Mirus TransIT-PRO® Transfection Kit; MIR5700). Follow manufacturers recommended protocol when using such reagents.***

### Reagents and Equipment

- Biological safety cabinet (Class II; Type A2; ISO 5)
- CHOZN® CHO K1 stock cell culture
- EX-CELL® CD CHO Fusion Growth Medium (prepared in Part I)
- 4 mm Electroporation Cuvettes (Sigma Z706094)
- T-25 $cm^2$ suspension cell (hydrophobic surface treated) culture flasks (Greiner Bio-one 690195 and 658195 or equivalent)
- Plasmid DNA containing a gene expression cassette for the protein of interest, as well as an expression cassette for the appropriate selection marker (recommended plasmid concentration ~1µg/µl)

***Note: Plasmid DNA preparations should be sterile (via ethanol precipitation) and re-suspended in sterile water with no salts or buffers.***

- 15 mL and 50 mL sterile conical centrifuge tubes (Corning 430052 and 430290 or equivalent)
- Sterile pipettes
- Centrifuge
- 70% isopropanol
- Sterile microfuge tubes (1.5 mL Eppendorf® or equivalent)
- Automated cell counter or hemocytometer
- $CO_2$ incubator (5% $CO_2$, 37 °C, humidified)
- Bio-Rad Gene Pulser® or similar electroporation instrument

### Procedure

i) (24 hours before transfection) Cell culture preparation.

   a. Inoculate a flask of CHOZN® CHO K1 cells (from the stock cell culture) to a final cell density of 5e6 cells/mL.

ii) Electroporation set-up (day of transfection).

a. Prepare Growth Medium (see Part I)

***Note: Growth Media is used as the transfection and recovery medium.***

b. Label the appropriate number of T-25 $cm^2$ suspension cell culture flasks (one per transfection).

c. Aseptically add 5.0 mL Growth Medium to each flask.

iii) Label and place electroporation cuvettes on ice to chill.

iv) Label sterile microfuge tubes for mixing cell suspensions with DNA.

v) Prepare cells for electroporation as follows:

a. Aseptically remove a cell culture sample from the flask and count by trypan blue exclusion using a hemocytometer or an automated cell counter. A minimum viability target of >90% must be achieved to proceed with electroporation.

b. Calculate the appropriate volume of stock culture needed for the transfections (6.25e6 cells/transfection).

Aseptically transfer calculated volume of stock culture to an appropriately sized sterile conical centrifuge tube.

c. Centrifuge at 220 rcf for 5 minutes at 15–25 °C.

d. Carefully aspirate off the supernatant without disturbing the cell pellet.

e. Re-suspend cell pellet in the Growth Medium (1.0 mL medium per transfection).

vi) Electroporation

a. For each electroporation, mix 0.8 mL of cell suspension (~5e6 total cells) with desired amount of DNA in a sterile microfuge tube. 30–50 µg of plasmid DNA is recommended for each transfection (the volume of DNA in an individual transfection should not exceed 50 µl).

b. Transfer the DNA/cell mix to the chilled electroporation cuvette, and electroporate using the following settings:

| Voltage | Capacitance | Pulse |
|---|---|---|
| 300 V | 950 µF | Exponential Decay |

c. Transfer ~0.6 mL of each electroporation condition to the 5 mL Growth Medium in the prepared T-25 $cm^2$ suspension cell culture flask (try not to transfer the white cell debris).

d. The transfected pool may now be placed under the appropriate selection procedure.

## Part VI: Single Cell Cloning

ExCell CHO cloning medium is designed for robust cloning of several cell types, including ECACC K1. Conditions and supplements may differ depending upon the selection strategy applied to the stable pool. Cloning outgrowth may be improved by supplementing 20% conditioned media (see Appendix) to the C6366.

## Part VII: Fed Batch Assay

The ExCell Advanced media and feed have been designed for high expression of recombinant therapeutics produced in multiple CHO cell types. This media is specifically optimized for fed batch stages of development.

## Appendix: Conditioned Media Preparation

### Purpose

This protocol describes procedures for the production of conditioned cell culture medium for single cell cloning by limiting dilution. Conditioned medium has been shown to be beneficial in single cell cloning processes by providing the clones with the beneficial nutrients and/or factors that are secreted by exponentially growing healthy cells.

### Reagents and Equipment

- Cells: Stock culture CHOZN CHO K1 (in exponential growth phase)
- Growth Media: EX-CELL® CD CHO Fusion Medium with 4 mM L-glutamine added
- Sterile shaker flask (determine appropriate volume)
- Sterile centrifuge tubes (determine appropriate volume)
- Sterile 0.2 µm Millipore Steriflip® filter apparatus or equivalent
- Sterile pipettes
- Water bath at 37 °C
- Centrifuge
- Automated cell counter or hemocytometer
- Biological safety cabinet (Class II; Type A2; ISO 5)
- $CO_2$ incubator (5% $CO_2$, 37 °C, humidified)
- Orbital shake plate
- 70% isopropanol

### Procedure

i) Set-up of production culture

a. Determine desired volume of production culture and prepare an appropriately size shaker flask.

b. Aseptically remove a cell culture sample from the stable pool stock culture and count by trypan blue exclusion using a hemocytometer or an automated cell counter.

c. Calculate amount of cells required from the stock culture and the volume of fresh Growth Medium required to achieve an initial cell density of 10e6 cells/mL.

d. Aseptically transfer the calculated volume of cells from the stock culture into to a sterile centrifuge tube.

e. Centrifuge cells at 220 × g for 5 minutes at 15-20 °C.

f. Carefully aspirate off the supernatant without disturbing the cell pellet.

g. Gently re-suspend the cell pellet in the appropriate volume of fresh Growth Medium.

h. Aseptically remove a cell culture sample from the production culture and count by trypan blue exclusion using a hemocytometer or an automated cell counter.

i. The initial viable cell density should be 0.9-1.2e6 cells/mL.

j. Place the culture in the incubator (37 °C, 5% $CO_2$, 80% humidity) on an orbital shaker set at 125 rpm, for 24 hours (± 4 hours).

ii) Production Culture Harvest (24 hours post culture initiation)

a. Aseptically remove a sample from the conditioned medium production culture and count by trypan blue exclusion using a hemocytometer or an automated cell counter.

b. Transfer culture to centrifuge tube(s).

c. Centrifuge at 2440 × g for 5 minutes.

d. Transfer the clarified medium (conditioned medium) to sterile 50 mL conical tube(s). Be careful not to disturb cell pellet.

e. Filter the clarified medium using a 0.2 µm Steriflip® filter device or equivalent.

f. Label filtered conditioned medium with Cell Line ID, “Conditioned Medium”, and expiration date (one week from the harvest date).

iii) Storage

a. Conditioned medium may be stored for up to 7 days at 2-8 °C.

b. Do NOT freeze conditioned medium.

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