

## BalanCD CHO Growth A

Catalog #	Product	Size
91128	BalanCD CHO Growth A	1 L Liquid
94120		10 L and 100 L Powder
		Additional package sizes are available upon request.

### Intended Use

For research or further manufacturing use only. BalanCD CHO Growth A is a chemically defined medium optimized for the production of recombinant proteins and monoclonal antibodies in Chinese Hamster Ovary (CHO) cells. This medium is designed to be used in conjunction with any feed medium; however, it achieves maximum performance with BalanCD CHO Feed 4.

### Product Description

BalanCD CHO Growth A is provided without L-Glutamine to extend shelf life. The recommended L-Glutamine concentration to add is 4-8 mM (20-40 mL/L). The medium should be used without L-Glutamine if the GS selection system is being used. Glucose should be monitored during culture, additional supplementation may be required. BalanCD CHO Growth A has been designed without Hypoxanthine and Thymidine for use with the dihydrofolate reductase (dhfr) and other selection systems. The medium contains no protein hydrolysates or any other undefined components. This medium contains no antibiotics or antimycotics.

### Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis which is available at [www.irvinesci.com](http://www.irvinesci.com) or upon request.

### Shipping

This product is shipped at room temperature. Upon receipt, store immediately at 2-8°C.

### Storage Instructions and Stability

Handle using aseptic techniques to avoid contamination. Store liquids at 2-8°C, away from light. Un-opened liquid medium (Cat #91128) is stable for 12 months, when properly stored. Store powders dry at 2-8°C. Un-opened powder medium (Cat #94120) is stable for 36 months, when properly stored. Do not use after the assigned expiration date. Do not use any bottle of medium which shows evidence of particulate matter or cloudiness.

# Directions for Use

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## Adaptation

### SUPPLEMENT FOR BALANCD CHO GROWTH A

BalanCD CHO Growth A can be supplemented with Anti-Clumping Supplement (Catalog #91150). Anti-Clumping Supplement is animal component-free and enzyme-free, and may be added to cultures if the cells start to aggregate. If severe cell aggregation is observed, continue passaging cells with Anti-Clumping Supplement. Anti-Clumping Supplement is designed for use at a dilution between 1:1000 (1 mL/L) and 1:100 (10 mL/L) depending on degree of clumping.

**Note:** *If this supplement is added, it must be eliminated from the culture media prior to transfection, as this supplement will completely inhibit transfection via cationic liposomes or cationic polymers such as polyethylenimine. Cells must be spun down and re-suspended in transfectory media without Anti-Clumping Supplement before proceeding with transfection.*

### I. Direct Adaptation from Serum-Supplemented Media to BalanCD CHO Growth A

In most cases, CHO cells may be subcultured from a serum-supplemented medium (e.g., Ham's F-12/DME + 10% FBS) directly into BalanCD CHO Growth A.

1. Dispense BalanCD CHO Growth A medium into a culture vessel and equilibrate to 37°C and 5% CO<sub>2</sub>.
2. Passage CHO cells from serum-supplemented culture into BalanCD CHO Growth A at 3x10<sup>5</sup> 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 37°C and 5% CO<sub>2</sub> until the viable cell density reaches 1x10<sup>6</sup> viable cells/mL.
4. Subculture into fresh BalanCD CHO Growth A medium at 3x10<sup>5</sup> viable cells/mL starting density.
5. Maintain cells in BalanCD CHO Growth A for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

### II. Sequential Adaptation from Serum-Supplemented Media to BalanCD CHO Growth A

Sequential adaptation may be used if direct adaptation is troublesome.

1. Dispense the original serum-supplemented medium and BalanCD CHO Growth A medium in a 3:1 ratio into an appropriate culture vessel and equilibrate to 37°C and 5% CO<sub>2</sub>.
2. Passage CHO cells from serum-supplemented culture into 3:1 BalanCD CHO Growth A at 3x10<sup>5</sup> 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 37°C and 5% CO<sub>2</sub> until the viable cell density reaches 1x10<sup>6</sup> viable cells/mL.
4. Subculture at 3x10<sup>5</sup> viable cells/mL starting density into fresh medium prepared in a 2:1 ratio of original serum-supplemented medium to BalanCD CHO Growth A.
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 Growth A. 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 Growth A for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

## PREPARATION FROM POWDER (Catalog No. 94120)

1. Add 23.72 g/L powder medium to WFI or deionized/distilled water in an appropriately sized container.
2. Mix the solution until the powder is well dissolved (the solution may still appear cloudy at this point). *Note: For a 1 L batch size, approximate stirring time is 30 minutes.*
3. Add 2.2 g/L sodium bicarbonate to the solution. Mix until sodium bicarbonate is completely dissolved. *Note: For a 1 L batch size, approximate stirring time is 10 minutes.*
4. Adjust pH to 6.8 to 7.2 by choosing **one** of the options below:
  1. Stir solution until pH reaches 6.8 to 7.2 for approximately 60-90 minutes for a 1 L batch size. Closely monitor pH and do not over stir as pH can drift past the target range.
  2. Add approximately 0.85 mL sodium hydroxide (5N) to raise the pH to 6.8 to 7.2 for a 1 L batch size. Closely monitor pH while adding sodium hydroxide as pH can drift past the target range.*Note: Mixing time and amount of sodium hydroxide addition can vary based on configuration of vessel and volume.*
5. Measure osmolality of the solution. Expected final osmolality is 290-310 mOsm/kg (adjust with sodium chloride, if necessary).
6. Sterile filter through a 0.2 µm filter membrane into sterile vessel.
7. Store at 2-8°C, in the dark for up to 1 year.

## CRYOPRESERVATION

Viable cell banks may conveniently be created by freezing cells in 90% BalanCD CHO Growth A + 10% DMSO. No other additions are necessary.

### Freezing

1. Use cultures that are in logarithmic growth with high viabilities (>85%).
2. Centrifuge cells for 5 minutes at 200 x g.
3. Resuspend in cold (2-8°C) 90% BalanCD CHO Growth A + 10% DMSO to a density of  $1 \times 10^7$  viable cells/mL.
4. Aliquot into sterile cryovials.
5. Gradually lower the temperature of the vials to below  $-80^{\circ}\text{C}$  at a rate of  $-1^{\circ}\text{C}/\text{minute}$ .
6. Store vials in liquid nitrogen freezer.

### Thawing

1. Thaw frozen vial rapidly in a  $37^{\circ}\text{C}$  water bath.
2. Transfer the cell suspension to a culture flask with fresh BalanCD CHO Growth A medium to achieve an initial cell density of  $3 \times 10^5$  viable cells/mL.
3. Incubate cultures at  $37^{\circ}\text{C}$  and 5%  $\text{CO}_2$  until the viable cell density reaches  $1 \times 10^6$  cells/mL.
4. Subculture into fresh BalanCD CHO Growth A medium at  $3 \times 10^5$  cells/ mL starting density.

## Related Products

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Catalog #	Product	Size
91127	BalanCD CHO Feed 1, Liquid	1 L
94119	BalanCD CHO Feed 1, Powder	10 L
91129	BalanCD CHO Feed 2, Liquid	1 L
94121	BalanCD CHO Feed 2, Powder	10 L
99471	BalanCD CHO Feed 3, Liquid	1 L
94118	BalanCD CHO Feed 3, Powder	10 L
94134	BalanCD CHO Feed 4, Powder	1 L and 10 L
91150	Anti-Clumping Supplement, Liquid	50 mL

BalanCD CHO Feed 1, 2, 3 and 4 are animal component-free, chemically defined feeds designed for the production of recombinant proteins in Chinese Hamster Ovary (CHO) cells.

## Technical support

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### CONTACT US

For more information or assistance, contact Customer Service at:

- Email: [tmrequest@irvinesci.com](mailto:tmrequest@irvinesci.com)
- Direct line: +1 800 577 6097

### WEBSITE RESOURCES

Visit the website at [www.irvinesci.com](http://www.irvinesci.com) for technical resources and information including:

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

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