

CELL | ST™

Cell culture media
For better lives

CELLiST™ CHO MX Medium

Shortcut to
Official Channel



CELLiST™ BASAL CHO MX Medium

Overview

CELLiST™ BASAL CHO MX cell culture medium provides everything your CHO cell line requires for stable, high-yield protein production. Developed through collaboration with KBI Biopharma and JSR Life Sciences, leveraging KBI's upstream cell culture process expertise, CELLiST™ BASAL CHO MX has been formulated for optimal performance. The ideal balance of amino acids and other nutrients in CELLiST™ BASAL CHO MX ensures adequate cell growth and maximum productivity. CELLiST™ BASAL CHO MX is completely chemically-defined and contains no animal- or plant-derived components, making it suitable for any CHO cell line.

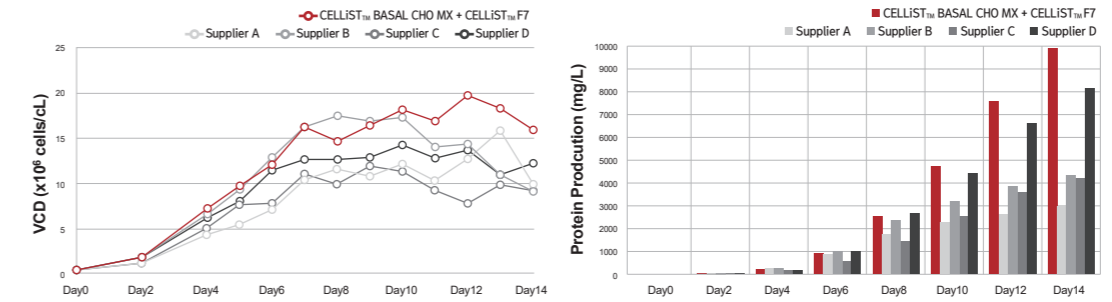


| CELLiST™ BASAL CHO MX Features | Benefits |
|--|--|
| Enriched with amino acids for enhanced cell growth. | Rapid cell proliferation, achieving higher cell densities faster. |
| Versatility across cell lines. | Compatible with a wide range CHO cell line, including CHO-K1, CHO-GS, CHO-S and their derivatives. |
| Chemically defined, protein-free medium with no animal-derived components. | Reduces risk of viral contamination, ensures batch consistency. |
| Supplied in fine powder form. | Easy to dissolve and allows for prolonged shelf life and ease of transportation. |
| Available in test samples and bulk sizes. | Flexibility according to usage requirements. |
| Suitable for batch, fed-batch, and perfusion cell cultures, at any scale. | Versatility across cell culture processes and scales. |

Cell Culture Performance

Comparative studies show superior culture growth and productivity with the CELLiST™ BASAL CHO MX + CELLiST™ F7 media combination. In a fed-batch process using CHO-K1 and CHO-S cell lines expressing IgG1 antibody, our media outperforms top global competitors in viable cell density and IgG titer.

CHO-K1



CHO-S

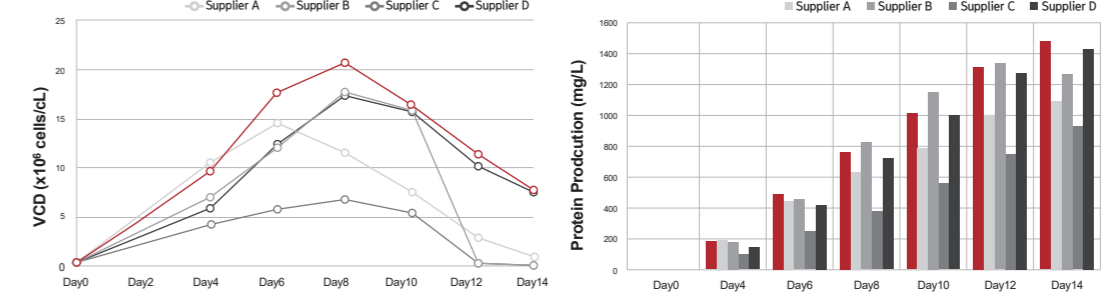


Figure 1: Fed-batch process was performed in an Ambr®15 system (CHO-K1) and 125 mL flasks (CHO-S), respectively. Feeding in CELLiST™ group was performed at a concentration of 6% (v/v) on days 4, 6, 8, 10, and 12. 'Supplier A, B, C and D' represent basal/feed media combinations from major media suppliers. Culture and feeding manners for supplier A, B, C and D were performed according to each manufacturer's recommendations.

Scalability

As can be seen below, CELLiST™ BASAL CHO MX medium is suitable for use in various culture scales from small scale microbioreactors to larger scale 200L bioreactors. CELLiST™ BASAL CHO MX medium shows consistent performance in terms of cell growth and productivity regardless of scale.

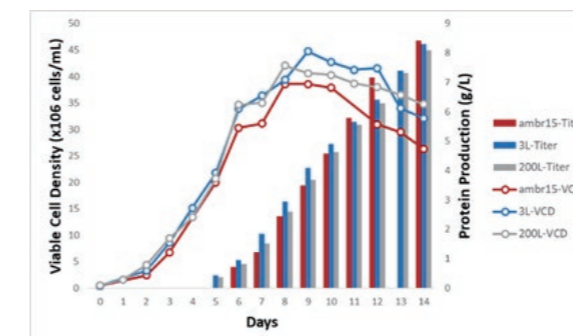


Figure 2. Viable cell density and IgG titer profiles during the 14 days of fed-batch culture. Three types of bioreactors were used: microbioreactors (Ambr®15), 3L bench-top reactor and 200L bioreactor.

Liquid Media Preparation

CELLiST™ BASAL CHO MX Medium Reconstitution (1L)

1. Prepare a suitable container and stir bar (magnetic bar). When preparing on a weight basis, measure the weight of the container and the stir bar.
2. Fill the container with approximately 90% volume (900 mL) of cell culture-grade water.
3. Add the entire contents of the pouch (i.e., 23.0 g) to the container. Rinse the pouch with a small amount of cell culture-grade water to wash the remaining product into the container.
4. Add 2.1 g of Sodium Bicarbonate.
5. Mix using magnetic stirrer for 20 minutes or until all powder is dissolved.
6. Add cell culture-grade water to a final volume of 1 L and mix the media for 10 minutes. Volume adjustments can also be made by weighing the solution.
7. Check the pH of the solution and adjust it to the range of 6.8 to 7.4 using either HCl or NaOH solutions as necessary.
8. Filter the liquid medium through a membrane filter with a pore size of 0.2 to 0.22 µm for sterilization inside a sterile biosafety cabinet.
9. Store in a refrigerated (2-8°C), dark environment until use.
10. Right before use, aseptically add L-glutamine or AminoStable™ (a final concentration of 2-6 mM is recommended), and add required growth factors such as insulin or IGF-1 into the solution.

Notes regarding cell passaging:

- a. It is highly recommended to passage the cells at least 3 times in their original medium, prior to transferring into the new CELLiST™ Medium.
- b. To reduce the stress faced by cells due to media switch process, it may help to add growth factors such as insulin or IGF-I (for example, 50 µg/L of LONG®R3 IGF-I).
- c. Cell adaptation into a new medium is very much dependent on the cell line and original medium being used. If direct switch ('direct adaptation') of cells from their original media to CELLiST™ results in unusual low viability and slow cell growth, sequential adaptation may be required (see the following section).



Cell Adaptation Strategy

1) Direct Adaptation

Most CHO cell lines can undergo direct adaptation to CELLiST™ media as follows.

1. Determine the cell concentration and viability of the culture. Cells should be in logarithmic growth phase (usually Day 3-5) with a viability of >90% prior to inoculation into new medium.
2. Seed cells at 0.3-0.5 x 10⁶ viable cells/mL in sterile culture vessels containing pre-warmed complete CELLiST™ BASAL medium.
3. Incubate at 37°C in a humidified incubator at 5% CO₂ on an orbital shaker platform rotating at the desired RPM (e.g. 100-150 RPM).
4. Passage (subculture) cells every 3-4 days or when viable cell density reaches >1.0 x 10⁶ cells/mL. Seed cells at densities of 0.3-0.5 x 10⁶ viable cells/mL.

2) Sequential Adaptation

Sequential adaptation of CHO cells into CELLiST™ media can be a favorable solution for cell lines facing challenges with direct adaptation (for example, exhibiting very slow cell growth). It is recommended to use a higher seeding density during the adaptation period, approximately (~0.5 x 10⁶ cells/mL). This sequential adaptation method allows a gradual adaptation of the cells to the new medium, achieved by sequentially increasing the relative volume of the new medium. The three-step adaptation procedure (100:0 → 50:50 → 0:100; ratio between Original-to-CELLiST™ medium) may be sufficient in most cases. However, for sensitive cell lines, it is recommended to perform a 5-step adaptation procedure, as described below:

| Ratio of Original vs. CELLiST™ medium | Seeding Density | Criteria for next stage |
|---------------------------------------|---------------------------|--|
| 100:0 | 0.3-0.5 x 10 ⁶ | Cell density 1-3 x 10 ⁶ ; Viability > 90% |
| 75:25 | 0.3-0.5 x 10 ⁶ | Cell density 1-3 x 10 ⁶ ; Viability > 90% |
| 50:50 | 0.3-0.5 x 10 ⁶ | Cell density 1-3 x 10 ⁶ ; Viability > 90% |
| 25:75 | 0.3-0.5 x 10 ⁶ | Cell density 1-3 x 10 ⁶ ; Viability > 90% |
| 0:100 | 0.3-0.5 x 10 ⁶ | Cell density 1-3 x 10 ⁶ ; Viability > 90% |

Note: Some cell lines require addition of growth factor for proper growth. The addition of growth factors, such as Insulin or IGF-I, can help the adaptation process in these cases that show extremely poor initial cell growth.

Other Products

| Products | Type | Features |
|----------------------|------------------------|--|
| CELLiST™ F7 | Feed medium | High-performance, all-in-one feed media that easily dissolves and remains stable at neutral pH, thanks to Ajinomoto Group's unique cysteine-stabilization technology. This enhances cysteine availability, boosting performance and enabling hassle-free, single-agent feeding |
| AminoSupplement Cys1 | Feed medium supplement | Performance-enhancing supplement for any single-agent feed media, improving culture performance and stability with Ajinomoto Group's unique cysteine stabilization technology. |
| AminoSupplement Cys2 | Feed medium supplement | Performance-enhancing supplement that eliminates the need for high-pH feeding in <u>dual-agent feed media</u> . Easily added at neutral pH, ensuring high stability and simplified processes. |
| Supplement Gly-L-Tyr | Feed medium supplement | Highly soluble tyrosine supplement that boosts tyrosine concentrations for any neutral pH feeding process, enhancing culture growth without solubility concerns. |



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