CELLiST™

Cell culture media

For better lives

Shortcut to
Official Channel

CELLiST™ BASAL3 & FEED2 Media

Overview

CELLiST provides an 'all in one' solution for all your biologics manufacturing needs. CELLiST BASAL3 growth medium, complimented with FEED2 supplement, provides everything your CHO cell line requires for stable, high yield protein production. CELLiST cell culture media products incorporate Ajinomoto's long history of know-how in the development and manufacturing of amino acids and amino acids-related products. CELLiST media are completely chemically-defined, animal origin-free, and is suitable for use with any CHO cell line.

Properties

- Chemically-defined, protein-free medium without any animal-derived components, hydrolysates, extracts or other undefined components.
- Suitable for all CHO cell lines including CHO-GS, CHO-K1, CHO-S and CHO-DG44.
- Suitable for batch, fed-batch, and perfusion cell cultures, at any scale.
- High performance in both cell growth and protein production
- Test samples as well as bulk size orders are available
- Flexible application for easily replacing any existing media platform
- Manufactured in a cGMP-complied factory
- CELLiST FEED media can be combined with any manufacturer's basal medium, though it is optimized to provide best performance when combined with CELLiST BASAL3

Specifications

CELLiST™ BASAL3

BASAL3 growth medium provides optimal balance of amino acids and other nutrients to ensure adequate cell growth and maximum productivity of your process. BASAL3 is completely chemically-defined and does not contain any animal origin components.

- Does not contain thymidine or hypoxanthine.
- Does not contain L-glutamine source.
- Does not contain sodium bicarbonate or poloxamer.
- Contains 10 g/L Glucose.
- Suitable for all CHO cell line including CHO-GS, CHO-K1, CHO-S, CHO-DG44.
- Recommended to use in combination with FEED2 supplement in fed-batch or perfusion culture, for optimal results.

CELLiST™ FEED2

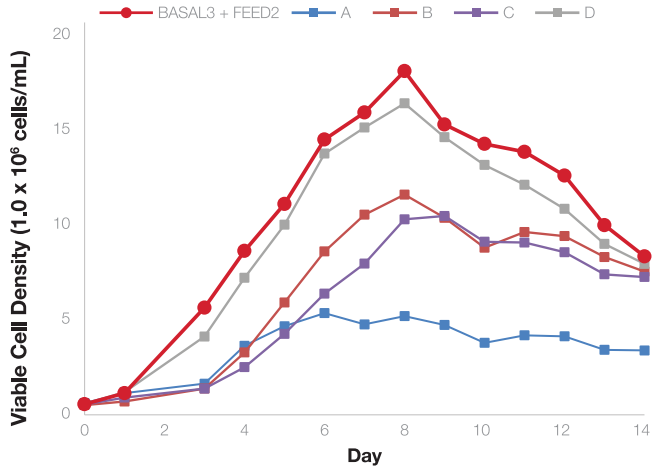
FEED2 supplement media should be added from day 3 or 4 of the culture in fed-batch process, or together with the basal medium in a perfusion processes. FEED2 is enriched with amino acids to support the rapid growth and production phases of the cell culture.

- Does not contain thymidine or hypoxanthine.
- Does not contain L-glutamine sources.
- Does not contain sodium bicarbonate or poloxamer.
- Does not contain L-Glucose or any other sugar source. Addition of L-Glucose should be optimized per cell line.
- Contains cysteine and tyrosine sources.
- FEED2 can be combined with any commercially-available basal medium, though for best performance it is recommended to be used together with CELLiST BASAL3.

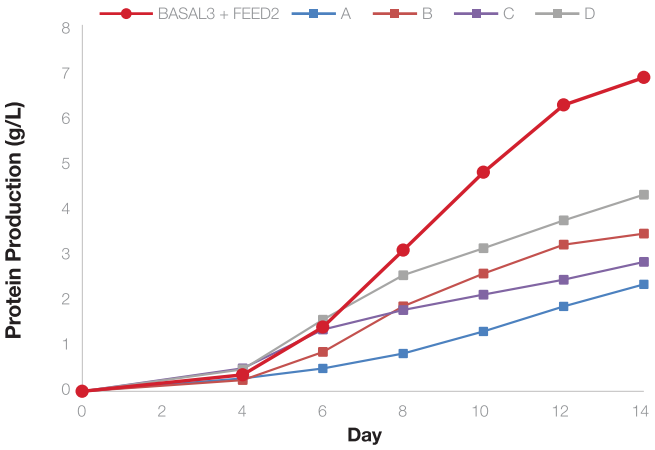
Media Performance

Cell culture performance results for CELLiST media and commercially-available media from other top global media manufacturing companies, are shown below. Fed-batch process was performed in an Ambr® 15 microbioreactor system, using a CHO-K1 line expressing IgG1 antibody.

Cell Growth



Protein Production



- A, B, C and D represent basal/feed combinations from major media manufacturers. Culture and feeding manner were performed according to each manufacturer's recommendation.

Liquid Media Preparation

BASAL3 Reconstitution (1 L)

1. Prepare a suitable container and stir bar (magnetic bar).

2. Fill the container with about 90% volume (900 mL) of cell culture-grade water (room temperature).

3. The total amount of this pouch (27.0 g) should be added to the container. Place a small amount of cell culture-grade water in the pouch to wash the remaining product into the container.

4. Add 1 g of Poloxamer 188 (Pluronic F-68) and 1.8 g of Sodium Bicarbonate.

5. Mix using magnetic stirrer for 20 minutes (until all powder is dissolved).

6. Add cell culture-grade water to final volume of 1 L and mix the media for 10 minutes. Volume adjustments can also be done by weighing (see table above).

7. Check pH to ensure a proper range of 6.8-7.2. If out of range, adjust pH using HCl or NaOH solutions.

8. Filter the medium in a biosafety cabinet, using a membrane filter with pore size of 0.2 to 0.22 µm in diameter.

9. Store in a refrigerated (2–8°C), dark environment until use.

10. Right before use, add L-glutamine or AminoStable™ to the solution (2-6 mM final concentration is recommended). If required, add growth factors such as insulin or IGF-I.

*Notes:
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. In order to reduce the stress faced by cells due to media switch process, it may help to add growth factor such as insulin or IGF-I (for example, 50 µg/L of LONG® R^3 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 Medium results in unusual low viability and slow cell growth, sequential adaption may be needed (see next page).

FEED2 Reconstitution (200 mL)

1. Prepare a suitable container and stir bar (magnetic bar). To ensure sufficient stirring, we recommend a container with a capacity of about 2–3 times the total prepared volume. When preparing on a weight basis, measure the weight of the container and the stir bar.

2. Fill the container with about 70% volume (140 mL) of cell culture-grade water (room temperature).

3. The total amount of this pouch (22.0 g) should be added to the container. Place a small amount of cell culture-grade water in the pouch to wash the remaining product into the container.

4. Add glucose as needed.

5. Stir for about 30 minutes.

6. Add 1.04 mL of 8N NaOH solution.

7. Stir for at least 30 minutes or until the powder is completely dissolved.

8. Make sure the pH is between 6.6-7.0. If the pH is lower than 6.6, add 8N NaOH solution to adjust to 6.6-7.0. If the pH is higher than 7.0, the stirring time should be extended to allow complete dissolution of the medium components.

9. Adjust to the final volume (200 mL) with cell culture-grade water and stir until the solution is clear for approximately 15 minutes.

10. Under aseptic conditions (e.g., in biosafety cabinet), filter sterilization using a filter with a pore size of 0.20–0.22 µm.

11. Store in a refrigerated (2–8°C), dark environment until use.

Fed-batch Culture Strategy

Cell Adaptation

1) Direct Adaptation

Most CHO cell lines can undergo direct adaptation to CELLiST medium 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 10^6 viable cells/mL in sterile culture vessels containing pre-warmed complete CELLiST BASAL medium (for example, 30 mL per 125 mL shake flask).

3. Incubate at 37°C in a humidified incubator at 5% CO_2 on an orbital shaker platform rotating at 100-130 rpm.

4. Passage (subculture) cells every 3-4 days or when viable cell density reaches >1 x 10^6 cells/mL. Seed cells at densities of 0.3-0.5 x 10^6 viable cells/mL.

2) Sequential Adaptation

Sequential adaptation of CHO cells into CELLiST medium may be required only if direct adaptation proves problematic, such as exhibiting very slow cell growth. It is recommended to use higher seeding density during the adaptation period (~0.5 x 10^6 cells/mL). Sequential adaptation allows gradual adaptation for the cells to the new medium, by sequentially increasing the new medium used. Three-step adaptation procedure (100:0 → 50:50 → 0:100; ratio between Original:CELLiST medium) might be enough, but it is recommended, especially in the case of sensitive cell lines, to perform a 5-step adaptation procedure, as described in the table below:

Ratio of Original vs. CELLiST medium	Seeding Density	Criteria for next stage
100:00	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 factor, such as Insulin or IGF-I, can help the adaptation process in these cases that show extremely poor initial cell growth.

Guidelines for Feeding Manner Optimization with CELLiST™ Medium

In order to achieve optimal growth and productivity, it is recommended to follow feeding manner optimization as described below.

a. Day 0 (Inoculation)

(1) Prepare liquid media following CELLiST media preparation instruction.

(2) Inoculate cells to 30 mL of basal media at 0.3 x 10^6 cells/mL in 125 mL Erlenmeyer flasks.
(If required, add L-glutamine and supplement with growth factors (IGF-I or Insulin)

(3) After inoculation, perform measurement to confirm appropriate cell desntiy and viability.

b. From day 3 or 4 to final day

(1) Take samples on desired time points (for example, once every two days) to confirm viable cell density and to measure desired metabolites and protein titer.

(2) Add CELLiST FEED medium at feeding volume of 2%-6% every 2 days, starting from day 3 or 4 (see table below). Feeding volume should be optimized according to cell line.

(3) During cell culture process, add glucose in order to maintain the desired glucose level (for example, between 2-6 g/L, depending on cell line). Glucose can also be added to the feed media itself, at concentration of 70-100 g/L, depending on feeding volume (see FEED media preparation instructions)

Table for Feeding Manner Optimization

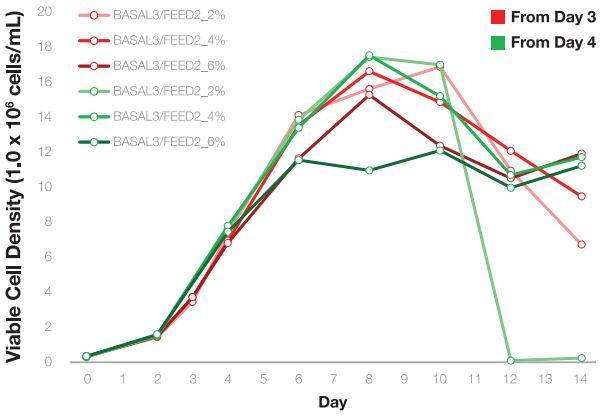
Culture day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
#1				2%		2%		2%		2%		2%			
#2					2%		2%		2%		2%		2%		
#3				4%		4%		4%		4%		4%			
#4					4%		4%		4%		4%		4%		
#5				6%		6%		6%		6%		6%			
#6					6%		6%		6%		6%		6%		

- Glucose concentration should be maintained the at 2-6 g/L

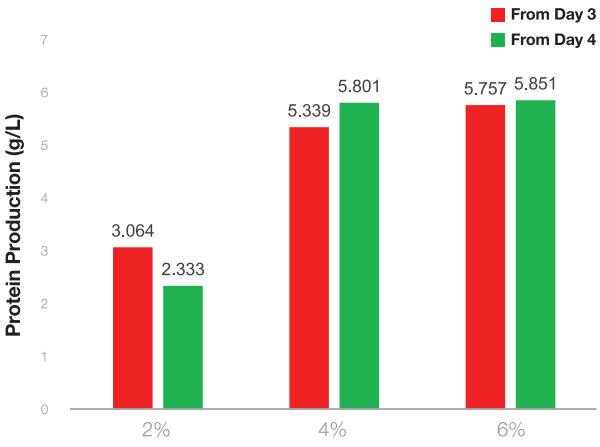
Culture performance for various feeding manners:

CHO-K1 cells were cultured in flask with 30 mL working volume on orbital shaker (115 rpm, 22 mm orbital diameter, 5% CO_2).

Viable Cell Density (VCD)



Protein Production



Technical Notes for Cell Culture

Growth factor supplementation

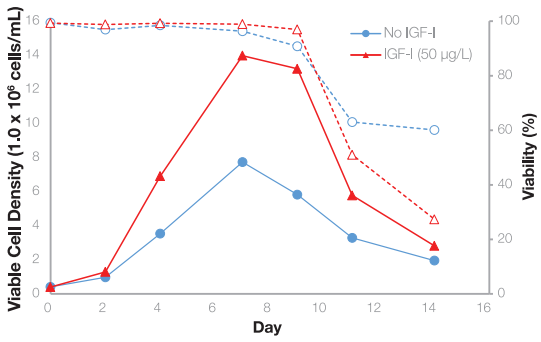
Growth factors such as insulin, insulin-like growth factor I (IGF-I) and its analogs, have all been shown to enhance cell growth, as well as antibody production, in CHO cells cultures (1-4).

LONG® R³ IGF-I human (Repligen) is a recombinant analog of IGF-I that has been specifically engineered for the enhancement of cell culture performance (5). It is more biologically potent *in vitro* than either insulin or native IGF-I and has been shown to significantly increase recombinant protein production (1, 2).

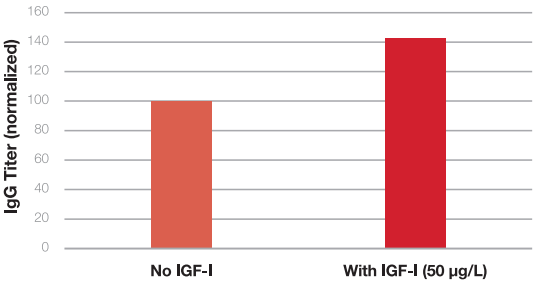
Here, we present the effect of the insulin-like growth factor LONG® R³ IGF-I human on both cell culture growth (viable cell density and viability) and antibody production (IgG titer). Although the magnitude of the effect depends on the specific cell clone, addition of growth factor can lead to an increase of up to 2-4 fold in cell culture performance. It can also assist in cell line adaptation when switching from one type of media to another.

The following graph shows the effect of growth factor addition on viable cell density, viability and IgG titer, in CHO-DG44 cell line. As can be seen from the graphs, addition of growth factor increased max viable cell density (VCD) by 80% and IgG titer by >40%.

Cell Culture Growth Profile



IgG titer



(1) Kim, D.Y., et al. (2005).
(2) Morris, A.E. and Schmid, J. (2000).
(3) Lim, U.M., et al. (2013).
(4) Miki H. and Takagi M. (2015)
(5) LONG® R³ IGF-I, Repligen

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