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

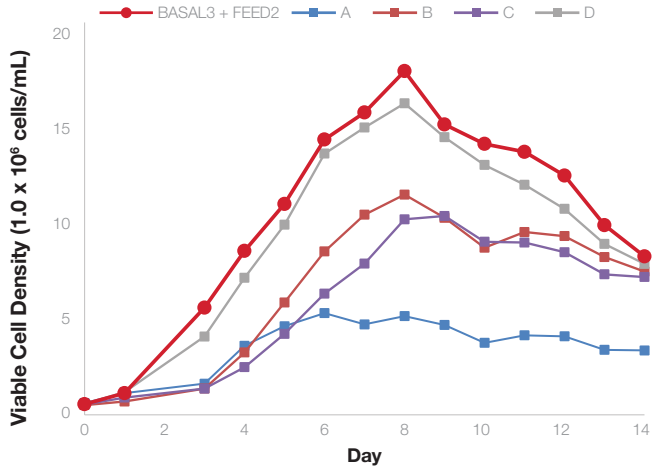
FEED2 supplement media should be added from day 3 or 4 of the culture in fed-batch process, or together with the basal medium in a perfusion processes. FEED2 is enriched with amino acids to support the rapid growth and production phases of the cell culture.

- 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.

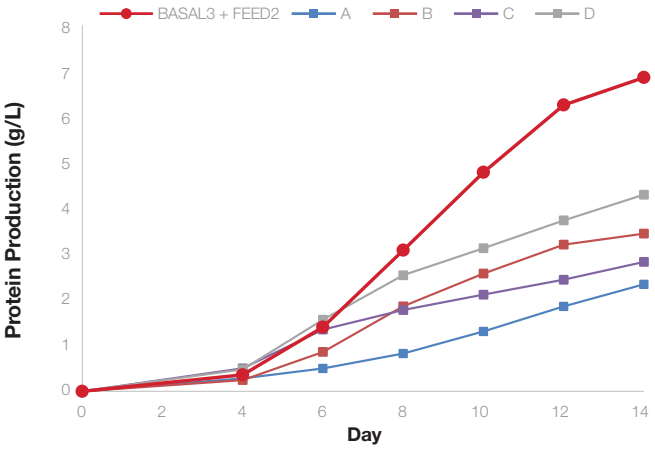
Media Performance

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. Add the entire contents of CELLiST powder medium (27.0 g) pouch to 900 mL room temperature deionized or distilled water (use a large enough mixing container such as beaker or conical flask). Rinse inside of package to remove all traces of the powder.

2. Add the necessary supplements, such as 1 g of Poloxamer and 1.8 g of Sodium Bicarbonate.

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

4. Add deionized or distilled water to final volume of 1 L and mix the media for 10 minutes.

5. Perform sterile filtering of the media in a clean bench, using a membrane filter with pore size of 0.22 µm in diameter (using an air pressure system). At this step, expected values of pH and osmotic pressure are as below;
pH : 5.8-6.4 (without any supplements)
Osmolarity: 255-305 mOsm/kg

6. Keep the prepared medium refrigerated (2°C to 8°C) until use.

7. Right before use, aseptically add L-glutamine source (for example, L-Alanyl-L-Glutamine from AJINOMOTO), and any growth factors (such as IGF-I), as required.

*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. Add the entire amount of the FEED2 medium powder (22.0 g) to a beaker or flask containing 140 mL of cell culture grade water (room temperature).

2. Rinse the package with a small amount of cell culture grade water to remove traces of powder, and add to the solution.

3. Mix for 30 minutes using magnetic stirrer. Make sure the mixing vortex is long enough that it almost reaches the bottom of the vessel. Do not heat the medium.

4. Add 1.04 mL of 8 N NaOH and continue mixing for additional 30 minutes.

5. Measure the pH to make sure it is in the desired range (6.5-7.0).

6. If needed, add the require amount of D-glucose at this step. (e.g. between 70-100 g/L glucose)

7. Add cell culture grade water to the solution to bring it to the final volume (200 mL) and continue mixing for 30 minutes, or until completely dissolved. To avoid evaporation, keep the vessel covered while mixing (using aluminum foil, for example).

8. Perform sterile filtering of the media in a clean bench, using a membrane filter with pore size of 0.22 µm in diameter (using an air pressure system). At this step, expected values of pH and osmotic pressure are as below;
pH : 6.5-7.0
Osmolarity: 420-470 mOsm/kg (1:2 dilution, without glucose)

9. It is recommended to aliquot the medium into tightly sealed conical tubes (e.g. 15 mL falcon tubes).

10. Keep the sterile medium refrigerated (2°C to 8°C) and use within two weeks.

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)

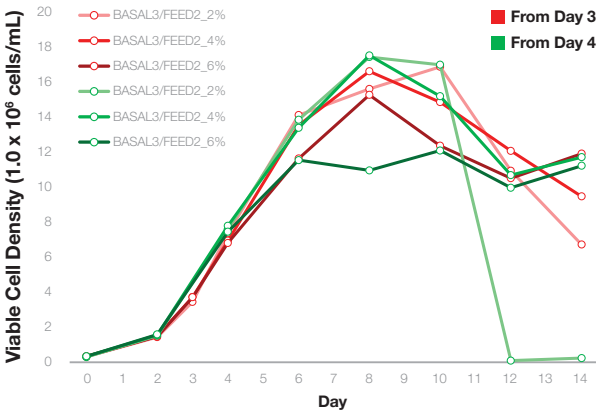
Table for Feeding Manner Optimization															
Culture day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
#1				2%		2%		2%		2%		2%			
#2					2%		2%		2%		2%		2%		
#3				4%		4%		4%		4%		4%			
#4					4%		4%		4%		4%		4%		
#5				6%		6%		6%		6%		6%			
#6					6%		6%		6%		6%		6%		

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

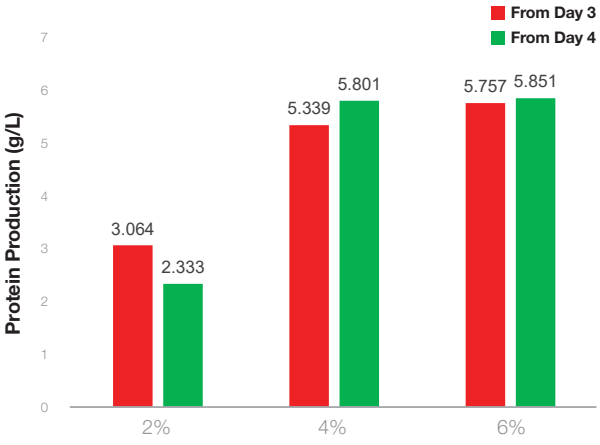
Culture performance for various feeding manners:

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

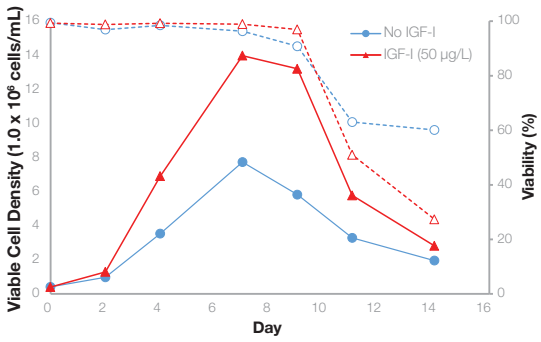
Growth factors such as insulin, insulin-like growth factor I (IGF-I) and its analogs, have all been shown to enhance cell growth, as well as antibody production, in CHO cells cultures (1-4).

LONG® R^3 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).

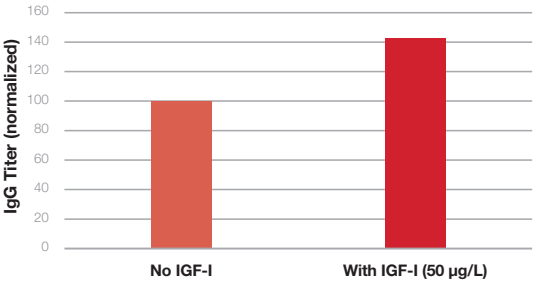
Here, we present the effect of the insulin-like growth factor LONG® R^3 IGF-I human on both cell culture growth (viable cell density and viability) and antibody production (IgG titer). Although the magnitude of the effect depends on the specific cell clone, addition of growth factor can lead to an increase 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® R^3 IGF-I, Repligen

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