ALLY CHO 300a & ALLY CHO 300b

Version 1.0

Introduction

The JSBio ALLY CHO 300a and ALLY CHO 300b supplements comprise chemically defined media devoid of any animal-origin components. Neither 300a nor 300b includes growth factors, hydrolysates, phenol red, or 2-mercaptoethanol, ensuring uniformity across batches. When coupled with CD CHO 031, CD CHO 050, and other media formulations, these supplements facilitate high-density, high-viability cultivation of CHO cells, as well as the production of recombinant proteins in CHO cells at elevated levels and with premium quality. This synergistic blend of media and supplements is compatible with a broad spectrum of CHO cell lines, notably including CHO-GS, CHO-K1, CHO-S, and CHO-DG44.

Product	Catalog No.	Form	Package Size
ALLY CHO 300a	99190-1426	Dry powder	5 L, 50 L, 100 L, Customized
ALLY CHO 300a	99190-24068	Liquid	500 mL, 1 L
ALLY CHO 300b	99169-1580	Dry powder	5 L, 50 L, 100 L, Customized
ALLY CHO 300b	99169-24069	Liquid	500 mL, 1 L

Component Information

Product	Glucose	L-Glutamine	Phenol Red	Sodium Bicarbonate
ALLY CHO 300a	with	without	without	without
ALLY CHO 300b	without	without	without	without

Safety Warning

Read the Material Safety Data Sheets (MSDS) and follow handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Intended Use

For research and further manufacturing use only.

Storage and Stability

Liquid media: The liquid media should be stored at 2–8°C and protected from light, shelf life of 3 months, the addition of other additives may affect storage conditions and shelf life. If precipitation or turbidity occurs, discontinue use.

Dry powder media: The dry powder media should be stored at 2–8°C and protected from light, shelf life of 12 months. This product is hygroscopic, once opened, please complete the liquid preparation within one month of opening the package.

ALLY CHO 300a Preparation Instructions

1. Fill the mixing container with purified water (room temperature) about 80% of the final volume, and start stirring.

2. Slowly add 181.08 g/L of dry powder medium. Mix until there is no dry powder on the surface of the solution. Mix for 30 minutes.
3. Adjust the pH to 8.2–8.4 with 5 M–10 M NaOH. Mix for 60 minutes.
4. Adjust the pH to 8.0–8.2 with 1 M–5 M HCl or NaOH. Mix for 10 minutes. The pH may rise 0.1 to 0.2 after sterile filtration.
5. Adjust the final volume with purified water. Mix for 10 minutes.
6. Filter the media using a membrane filter with 0.22 μm pore size immediately.

ALLY CHO 300b Preparation Instructions

1. Fill the mixing container with purified water (room temperature) about 60%–70% of the final volume, and start stirring
2. Slowly add 75.53 g/L of dry powder medium. Mix until there is no dry powder on the surface of the solution. Mix for 30 minutes.
3. Adjust the pH to 11.6–11.8 with 10 M HCl or NaOH. Mix for 30 minutes. The pH may rise 0.1 to 0.2 after sterile filtration.
4. Adjust the final volume with purified water. Mix for 10 minutes.
5. Filter the media using a membrane filter with 0.22 μm pore size immediately.

Culture Conditions

Basal Media: High-performance basal medium (CD CHO 031, CD CHO 050, CD CHO 051).

Feed Media: ALLY CHO 300a & ALLY CHO 300b.

Cell Line: CHO cell lines such as CHO-GS, CHO-K1, CHO-S and CHO-DG44.

Inoculation density:（0.8 -1.0）x 10^6 cells/mL

Culture Type: Suspension

Culture Vessel: TPP tubes/ Shaker flask/ Bioreactor

Recommended parameters for different culture vessels:

TPP tubes：	
Parameters	**Recommended value**
50 mL TPP tubes	Culture volume：10–30 mL
Shaker Speed	200rpm @ 50 mm orbital diameter
Culture Temperature	36-37°C*
CO_2 Concentration	5%
Relative Humidity	60% - 80% RH

Shaker flask：	
Parameters	**Recommended value**
125 mL Shaker flask	Culture volume：15–40 mL
250 mL Shaker flask	Culture volume：40–80 mL

500 mL Shaker flask	Culture volume：100–150 mL
1000 mL Shaker flask	Culture volume：200–300 mL
Shaker Speed	100-130rpm @ 50 mm orbital diameter
Culture Temperature	36-37°C*
CO_2 Concentration	5%
Relative Humidity	60%–80% RH

Bioreactor	
Parameters	**Recommended value**
Culture Temperature	36-37°C*
CO_2 Sparger	0.01 VVM
Air Sparger	0.01 VVM
O_2 Sparger	The initial setting is 0.02 VVM. It should be upregulated according to the increase of viable cell density.
pH	7.1 ± 0.2
DO	30%–50%

*The passaging temperature and the fed-batch temperature may differ. In general, the recommended passaging temperature is 36-37°C, and in fed-batch experiments the temperature can be lowered to a lesser extent during the process.

Usage Steps- Fed-batch

After continuous passaging in basal medium, proceed with the Fed-batch experiment when the cell count meets the required criteria.

1. Determine the cell density (× 10^6 cells/mL) and viability (%) using a cell counter and calculate the required volumes of JSBio CHO media and cell suspension based on an inoculation density of 0.8 - 1.2 × 10^6 cells/mL.
2. Based on the calculated volumes, withdraw the corresponding amounts of JSBio CHO media and cell suspension for passaging, inoculate the cells into new containers, and culture the cells according to the "Cultivation Conditions", add feed medium as per the experimental design.
3. Measure the cell density (× 10^6 cells/mL) and viability (%) daily, record the data, and plot the cell growth curve.
4. It is recommended to measure the metabolic parameters every second day from the second day, to add glucose according to the experimental design and to plot the metabolic curves.
5. Then days 8-10-12-14, under aseptic conditions, 1 mL of cell suspension was removed. harvest cells by centrifugation at 1300 × *g* (about 4500rpm) for 10minutes, store at -80°C.
6. The recommended culture cycle is 14 days. If cell viability is less than 60% or the protein yield reaches the set expression level during the experiment, the experiment can be stopped and the culture harvested; the yield can be analyzed and the yield curve plotted using ForteBio or similar equipment.

Fed-batch Culture

Combination	Base Media	Feed Media	Feeding Time (Day)	Add Amount (Add volume: add the working volume of the day)
A	JSBio basal media	ALLY CHO 300a & ALLY CHO 300b	3/ 5/ 7/ 9/ 11/ 13	ALLY CHO 300a: 4%/ 4%/ 4%/ 4%/ 4%/ 4% ALLY CHO 300b: 0.4%/ 0.4%/ 0.4%/ 0.4%/ 0.4%/ 0.4%
Inoculation density	（0.8 - 1.2）× 10^6 cells/mL			
Control	Base and feed media originally used by the customer			
Cultivation Conditions	Refer to "Culture Conditions" for the entire culture period, where the temperature should be maintained at 37°C. If a temperature reduction is required, refer to the original procedure. It is recommended to reduce the temperature to 33-35°C when the cell density exceeds 10 × 10^6 cells/mL.			
Note	Glucose should be controlled at 6 g/L. The critical concentration needed for the glucose addition is 3 - 4 g/L. The volume of feed media and glucose added can be calculated according to the actual working volume of the day.			

Related products

Product	Catalog No.	Form	Package Size
CD CHO 031	88031-585	Dry powder	5 L, 50 L, 100 L, Customized
CD CHO 031	88031-20090	Liquid	500 mL, 1 L