The User's Manual—Tobitec FB Pro

Basic Information Introduction

Product Introduction

Tobitec FB Pro is chemically-defined (CD) feed media, contains amino acids, vitamins, glucose, inorganic salts and trace elements, without protein, protein hydrolysate, growth factor and any animal-derived components. Tobitec FB Pro is an efficient feeding formula specially designed to improve the growth and product performance of CHO cells, which can meet the nutritional requirements of high-density cell culture and high product expression. Tobitec FB Pro in combination with Tobitec FA Pro can achieve high density stable cell culture and high level expression of recombinant proteins/antibodies.

Application Scope

Tobitec FB Pro can be applied to the fed batch culture of CHO cells, effectively improving the cell density and product expression. It can be used in the research and further manufacturing of protein/antibody related products, but cannot be directly used in the human body or used as a medication.

Shipping, Storage and Validity Period

Product	Catalog No.	Storage	Shipping	Validity period
Tobitec FB Pro	LQF02-B, Liquid	2°C ~ 8°C, Protect from light	2°C ~ 8°C, Protect from light	6 months
Tobitec FB Pro	DPF02-B, Powder	2°C ~ 8°C, Protect from light	2°C ~ 8°C, Protect from light	24 months

Protocol for Hydration of Powder Medium

1. Fill the mixing container with purified water (20 ~ 30°C) at 70% of the final volume.

2. Slowly add 94.50 g/L of powder medium with gentle stirring. Mix for 10 ~ 30 minutes.

3. Adjust the pH to 10.40 ~ 11.40 using 5M NaOH solution. Mix for 10 ~ 60 minutes.

4. Adjust the solution volume to 100% with purified water. Mix for 5 ~ 10 minutes.

5. Filter immediately the media with a 0.22 μm membrane filter.

Quality Index of Powder and Liquid Media

Product Index	Tobitec FB Pro (LQF02-B), Liquid	Tobitec FB Pro (DPF02-B), Powder
Appearance	Light yellow, clear liquid	Off-white powder
pH	11.0 ~ 11.6	10.4 ~ 11.4 (pre-filter)
Osmolality (mOsmol/kg)	180 ~ 280 (5-fold dilutions)	220 ~ 300 (5-fold dilutions)
Solubility	--	Dissolve well according to the protocol for hydration of powder medium
Sterility	Negative	--
Bioburden	--	Aerobic bacteria: < 200 CFU/g Molds and yeasts: < 50 CFU/g

Reference Fed Feeding Strategy

Feeding Strategy of CHO Fed Batch Culture

Application of Tobitec FB Pro in CHO Cell Stable Expression

1. Seed cells at 0.5×10^6 cells/mL into CHO basal media.

2. Starting from day D3, fed feeding according to the following feeding strategy:

Feeds/Time	D3	D5	D7	D9	D11	D13
Tobitec FA Pro / FB Pro	2/0.2%	2/0.2%	2/0.2%	2/0.2%	2/0.2%	2/0.2%
Tobitec FA Pro / FB Pro	3/0.3%	3/0.3%	3/0.3%	3/0.3%	3/0.3%	3/0.3%
Tobitec FA Pro / FB Pro	4/0.4%	4/0.4%	4/0.4%	4/0.4%	4/0.4%	4/0.4%
Glucose	When the glucose concentration is below 4 g/L, glucose is added at the final concentration of 6 g/L					

Suggestions:

It is suggested that the density of viable cells in the first feeding should be $4 \sim 6 \times 10^6$ cells/mL, and the feeding can be advanced or delayed according to the growth of cells.

In order to maintain high cell density and high viability during fed batch culture, it is recommended to lower the culture temperature when the viable cell density exceed 15×10^6 cells/mL.

Application of Tobitec FB Pro in CHO Cell Transient Expression

After CHO cell transient operation, culture cells under specified environmental conditions and fed according to the following feeding strategy:

Feeds/Time	D1	D4	D7
Tobitec FA Pro / FB Pro	2/0.2%	2/0.2%	2/0.2%
Tobitec FA Pro / FB Pro	3/0.3%	3/0.3%	3/0.3%
Tobitec FA Pro / FB Pro	4/0.4%	4/0.4%	4/0.4%
Glucose	When the glucose concentration is below 4 g/L, glucose is added at the final concentration of 6 g/L		
Glutamine	Control glutamine 2 ~ 6 mmoL/L after transfection or add 4 mmoL/L glutamine according to culture volume during feeding		