



The User's Manual—Tobitec FD

Basic Information Introduction

Product Introduction

Tobitec FD is chemically-defined (CD) feed media, containing amino acids, vitamins, glucose, inorganic salts and trace elements, without protein, protein hydrolysate, growth factors and any animal-derived components. Tobitec FD is an efficient feeding formula specifically designed to improve HEK293 cell growth and product production performance, which can meet the nutritional needs of high-density cell culture and high product expression. Tobitec FD combined with HEK293 CDM15 Pro, HEK293 CDM26 Pro and HEK293 CDM30 Pro could achieve high density stable cell culture and high level expression of recombinant proteins or antibodies and virus products.

Application Scope

Tobitec FD can be applied to the Fed-batch culture of HEK293 cells, effectively improving cell density and product expression. It is suitable for the development and production process of protein or antibody products, but cannot be directly used in the human body or as a drug.

Shipping, Storage and Validity Period

| Product | Catalog No. | Storage | Shipping | Validity Period |
|------------|-----------------|----------------------------------|---|-----------------|
| Tobitec FD | LQF01-D, Liquid | 2°C ~ 8°C, Protect from light | $2^{\circ}\text{C} \sim 8^{\circ}\text{C}$, Protect from light | 6 months |
| Tobitec FD | DPF01-D, 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 $(20 \sim 30^{\circ}\text{C})$ at 90% of the final volume.
- 2. Slowly add 190.00 g/L of powder medium with gentle stirring. Mix for 30 minutes.
- 3. Adjust the pH to $6.6 \sim 6.8$ using 5M NaOH solution. Mix for more than 30 minutes.
- 4. Adjust the solution volume to 100% with purified water. Mix for $5 \sim 10$ minutes.
- 5. Filter immediately the media with a $0.22 \mu m$ membrane filter.





Quality Index of Powder and Liquid Media

| Product Index | Tobitec FD (LQF01-D), Liquid | Tobitec FD (DPF01-D), Powder | |
|------------------------|------------------------------|--|--|
| Appearance | Light red, clear liquid | Off white powder | |
| pH | 6.6 ~ 7.4 | 6.6 ~ 7.4 (pre-filter) | |
| Osmolality (mOsmol/kg) | 300 ~ 350 (5-fold dilution) | 2350 (5-fold dilution) 300 ~ 350 (5-fold dilution) | |
| Solubility | | Dissolve well according to the protocol for hydration of powder medium | |
| Endotoxin (EU/mL) | < 3 | < 10 | |
| Sterility | Negative | | |
| Bioburden | | Aerobic bacteria: < 200 CFU/g Molds and yeasts: < 50 CFU/g | |

Reference Fed Feeding Strategy

HEK293 Fed-batch culture fed-feed strategy

Tobitec FD is used for protein expression of HEK293 cells

After the HEK293 transient operation, the cells were cultured under specified environmental conditions, refer to the following feeding strategies for feeding:

| Feeds/Time | D0 | D1 | D3 | | |
|------------|---|----|----|--|--|
| Tobitec FD | | 5% | 5% | | |
| Glucose | When the glucose concentration is below 3 g/L, glucose is added at the final concentration of 5 g/L | | | | |
| Glutamine | Control glutamine $2 \sim 6$ mmoL/L after transfection or add 4 mmoL/L glutamine according to the culture volume at the time of feeding | | | | |