

# ExCell Bio

## OptiVibro<sup>®</sup> CHO Serum-free Feed Medium TransExp CA06

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

### User Manual

Catalog Number CA000-N032

Catalog Number CA000-N033

Catalog Number CA000-N034

Catalog Number CA000-N035



## | PRODUCT DESCRIPTION

OptiVibro® CHO Serum-free Feed Medium TransExp CA06 is a state-of-the-art, animal component-free and chemically-defined medium that is specifically designed for the high-density culture transfection of suspended CHO-K1 and CHO-S etc., especially suitable for ExpiCHO-S cells. This product does not require additional glutamine.

It can be used together with OptiVibro® CHO Serum-free Medium TransExp CE06 (Catalog no. CE000-N05#) to promote protein production.

## | SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Product Name	Cat.#	Specification	Storage	Transportation	Shelf Life
OptiVibro® CHO Serum-free Feed Medium TransExp CA06	CA000-N032	100 mL Liquid	2-8 °C Protect from light	<10 °C Protect from light	12 months
	CA000-N033	1000 mL Liquid			
	CA000-N034	1 L Powder	2-8 °C Dark and dry	<10 °C Protect from light	24 months
	CA000-N035	10 L Powder			

## | PERFORMANCE, APPLICATION AND RESTRICTION

1. Please make sure to store the cell culture medium in a light-protected environment, avoid fluorescent lamps or other lamplight exposure, and better to use colored packaging bags in the refrigerator or warehouse.
2. During the transportation of products, try to avoid the impact of fluorescent lamps or other lamplight exposure on the appearance of the product, resulting in appearance discoloration.
3. During the transportation of the product to the clean area, it is essential to carry out a cleaning and sterilization process. The sterilization method may involve disinfectant wiping, and not utilize UV irradiation for sterilization.

4. Note: When passing through transfer windows equipped with UV sterilization, remember to proactively switch off the UV lamp inside the transfer window.

## | INSTRUCTION FOR USE

### **Medium preparation**

1. Measure 60% of the final volume WFI or distilled water in a clean vessel.
2. Add 157.64 g/L OptiVibro® CHO Serum-free Feed Medium TransExp CA06 powder slowly to the water, mix for 60 minutes.
3. Adjust the pH to 8.50-8.60 with 5 N NaOH (about 34 mL). After adjusting, continue stirring for an additional 60 minutes.
4. Adjust the pH to 7.00-7.10 with 6 N HCl (about 9 mL). After adjusting, continue stirring for an additional 10 minutes.
5. QS to final production volume and mix for 10 minutes.
6. Measure the osmotic pressure. The osmotic pressure range should be within 280-320 mOsm/kg (5-fold dilution).
7. Sterilize immediately by membrane filtration (< 0.22 microns), and store at 2 to 8 °C .

### **Cell Culture**

Suggested culture condition, Temperature:37°C, RH:80%, CO<sub>2</sub>:5%, 120rpm. According to the cell growth, it can be passed every 2-4 days when the living cell density reaches 4.0-6.0×10<sup>6</sup> cells/mL, and the passage density is 0.2-0.3×10<sup>6</sup> cells/mL.

### **Pre-transfection Preparation**

#### **1. Cell Maintenance**

- (1) Perform at least three consistent passages following cell recovery.
- (2) Ensure cell viability exceeds 90% before proceeding with transfection.

#### **2. Cell Seeding (Day -1)**

- (1) Resuspend cells in fresh OptiVibro® CHO Serum-free Basal Medium TransExp CE06.
- (2) Initial seeding density: 3.5 × 10<sup>6</sup> cells/mL.

**[Note]** : Omitting centrifugation may result in reduced protein expression.

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## **Transfection Procedure (Day 0)**

### **Cell Preparation**

1. Adjust cell suspension to 18 mL using fresh OptiVibro® CHO Serum-free Basal Medium TransExp CE06.
2. Target parameters:
  - (1) Total cell count:  $1.2 \times 10^8$  cells.
  - (2) Final transfection density:  $6 \times 10^6$  cells/mL in 20 mL culture.

### **PEI/DNA Complex Formation**

#### 1. Transfection Parameters:

- (1) Culture volume: 20 mL.
- (2) Cell density:  $6 \times 10^6$  cells/mL.
- (3) DNA concentration: 1.5 µg/mL.
- (4) DNA:PEI ratio: 1:4.

#### 2. Complex Preparation Steps:

##### a) PEI Max Solution:

- (1) Dilute 120 µg PEI Max in OptiVibro® CHO Serum-free Basal Medium TransExp CE06 to 1 mL.
- (2) Incubate at room temperature for 5 minutes.

##### b) DNA Solution:

- (1) Dilute 30 µg DNA in OptiVibro® CHO Serum-free Basal Medium TransExp CE06 to 1 mL.

##### c) Complex Formation:

- (1) Add PEI Max solution to DNA solution.
- (2) Mix thoroughly.
- (3) Incubate at room temperature for 10 minutes.

#### 3. Transfection

- (1) Add 2 mL PEI/DNA complex to cell suspension.
- (2) Maintain continuous flask agitation during addition for optimal mixing.

### **Post-transfection Process**

#### 1. Initial Feed (18-24 hours post-transfection):

- (1) Add 5% volume OptiVibro® CHO Serum-free Feed Medium TransExp CA06.

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(2) Supplement with glucose to final concentration of 22 g/L.

(3) Shift culture temperature to 33°C.

2. Feed Strategy Options:

a) Basic Strategy (for standard protein yields):

(1) Single feed at 18-24 hours post-transfection (as described above).

(2) Recommended when simplified operation is preferred.

b) Enhanced Strategies (for maximizing protein yields):

(1) Option 1: Two-step feeding.

Add 5% OptiVibro® CHO Serum-free Feed Medium TransExp CA06 on D1 and D3.

(2) Option 2: Three-step feeding.

Add 5% OptiVibro® CHO Serum-free Feed Medium TransExp CA06 on D1, D3, and D6.

**【Note】** : Additional feeding strategies should be implemented based on protein yield requirements and operational considerations.

3. Optional Enhancement

(1) Add OptiVibro® protein expression enhancer (Catalog no. M10141#) on D1.

(2) Refer to Table 1 for specific addition volumes.

**Harvest**

1. Standard harvest time: Day 10 post-transfection.

2. Earlier harvest may be considered based on:

(1) Target protein characteristics.

(2) Cell viability status.

**【Note】**

The aforementioned transfection techniques are intended for informational purposes solely. To achieve the most favorable transfection parameters for various CHO cells, a Design of Experiments (DoE) approach can be utilized for establishing the optimal experimental design, encompassing cell density, DNA quantity, and DNA to PEI ratio.

**Table 1. Recommended dosage for various transfection specifications**

Cell culture vessel	125 mL	500 mL	1 L	
Amount of cell (x 10 <sup>6</sup> cells/mL)	12	600	1200	cell density 6x 10 <sup>6</sup> cells/mL
OptiVibro <sup>®</sup> CHO Serum-free Basal Medium TransExp CE06 (mL)	18	90	180	Initial culture volume
DNA diluent (mL)	1	5	10	
PEI diluent (mL)	1	5	10	
DNA (µg)	30	150	300	DNA:PEI=1:4
PEI Max (µg)	120	600	1200	
OptiVibro <sup>®</sup> CHO Serum-free Feed Medium TransExp CA06 (mL)	1	5	10	5% of the initial transfection volume
OptiVibro <sup>®</sup> Protein expression enhancer(mL)	0.02	0.1	0.2	0.1% of the initial transfection volume
Final culture system (mL)	~21	~105	~210	

**Table 2. Related product number**

Product Name	Cat.#	Specification
OptiVibro <sup>®</sup> CHO Serum-free Medium TransExp CE06	CE000-N052	1 000mL Liquid
	CE000-N053	1 L Powder
	CE000-N054	10 L Powder
	CE000-N055	100 L Powder
OptiVibro <sup>®</sup> Glucose Solution	M101382C	100 mL Liquid
OptiVibro <sup>®</sup> Protein Expression Enhancer	M101412C	1 mL Liquid
	M101413C	5 mL Liquid

## | DISCLAIMER

1. The product should be used according to the instructions in the manual. If the experimenter fails to operate according to the instructions, our company will not be responsible for any deviation in product performance caused by this.

2. The product is only used for scientific research and commercial production, and is not suitable for clinical diagnosis and treatment. Otherwise, all consequences arising shall be borne by the experimenter, and our company shall not be responsible.