EIV22025E

ExCell Bio

OptiVitro® CHO Serum-free Feed Medium

TransExp CA06

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	CA000-N031
	CA000-N032
	CA000-N033
	CA000-N034
	CA000-N035

PRODUCT DESCRIPTION

OptiVitro® CHO Serum-free Feed Medium TransExp CA06 is a state-of-the-art, animal component-free and chemically-defined feed that is specifically designed for the high-density 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 OptiVitro® CHO Serum-free Medium TransExp CE06 (Catalog Number: CE000-N052) to promote protein production.

SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Product Name	Cat. #	Specification	Storage	Transportation	Shelf Life
OptiVitro® CHO Serum-free Feed Medium TransExp CA06	CA000-N031	50 mL Liquid	2-8 ℃ Protect From light.	<10 ℃ Protect From Light.	12 months
	CA000-N032	100 mL Liquid			
	CA000-N033	1000 mL Liquid			
	CA000-N034	1 L Powder	2-8 ℃ Dark and dry.	<10 ℃ Protect From Light.	24 months
	CA000-N035	10 L Powder			

HANDLING RECOMMENDATIONS

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 the product, it should be kept away from light. This is to prevent the product from being affected by the irradiation of fluorescent lamps or other light sources, which may lead to discoloration.
3. During the transportation of the product to the clean area, it is essential to carry out a cleaning process. The cleaning method may involve disinfectant wiping, and not utilize UV irradiation.

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

Medium preparation

1. Measure 60% of the final volume WFI or cell culture grade water in a clean vessel.
2. Add 157.64 g/L OptiVitro® 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 and record the final pH and osmolality. pH should be 6.90 to 7.50. Osmolality should be 280 to 320 mOsm/kg (dilute 5 times the measured value).
7. Sterilize immediately by 0.22μm membrane filtration. Store the reconstituted medium protected from light at 2°C to 8°C until use.

Cell Culture

Suggested culture condition, Temperature:37°C, RH:80%, CO_2:5%, 120 rpm. According to the cell growth, it can be passed every 2-4 days when the living cell density reaches 4.0-6.0×10^6 cells/mL, and the passage density is 0.2-0.3×10^6 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 OptiVitro® CHO Serum-free Basal Medium TransExp CE06.
   2) Seeding density: 3.5×10^6 cells/mL.

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

Transfection Procedure (Day 0)

Cell Preparation

1. Adjust cell suspension to 18 mL using fresh OptiVitro® CHO Serum-free Basal Medium TransExp CE06.
2. Target parameters:
   1) Total cell count: 1.2×10^8 cells.
   2) Final density after transfection: 6.0×10^6 cells/mL in 20 mL culture.

PEI/DNA Complex Formation

1. Transfection Parameters:
   1) Culture volume: 20 mL.

2) Cell density: 6.0×10^6 cells/mL.
3) DNA concentration: 1.5 μg/mL.
4) DNA:PEI ratio: 1:4.

2. Complex Preparation Steps:

PEI Max Solution:

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

DNA Solution:

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

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 18mL cell suspension.
2) Maintain continuous flask shaking during addition for optimal mixing.

Post-transfection Process

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

1) Add 5% volume OptiVitro® CHO Serum-free Feed Medium TransExp CA06.
2) Supplement with glucose to final concentration of 22 g/L.
3) Shift culture temperature to 33℃.

2. Feed Strategy Options:

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.

Enhanced Strategies (for maximizing protein yields):

1) Option 1: Two-step feeding.

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

1) Option 2: Three-step feeding.

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

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

3. Optional Enhancement

1) Add OptiVitro® protein expression enhancer (Catalog Number: M101412C) 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 volume

Cell culture vessel	125 mL	500 mL	1 L	
Amount of cell ($\times 10^6$ cells)	120	600	1200	cell density 6.0×10^6 cells/mL
OptiVitro® 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	
OptiVitro® CHO Serum-free Feed Medium TransExp CA06 (mL)	1	5	10	5% of the initial transfection volume
OptiVitro® 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 products

Product Name	Cat. #	Specification
OptiVitro® CHO Serum-free Medium TransExp CE06	CE000-N052	1000 mL Liquid
	CE000-N053	1 L Powder
	CE000-N054	10 L Powder
	CE000-N055	100 L Powder
OptiVitro® Glucose Solution	M101382C	100 mL Liquid
OptiVitro® Protein Expression Enhancer	M101412C	1 mL Liquid
	M101413C	5 mL Liquid

DISCLAIMER

1. Use the product according to the manual instructions. Deviations from these instructions are at the user's risk, and our company will not be responsible for any resulting product performance deviations.

2. This product is for scientific research and commercial production only and is not intended for clinical diagnosis or treatment. Users assume all risks for unauthorized use, and our company shall not be responsible for any consequences.