EmCD HEK293 Plus Feed medium User Guide

P30310

Product Description

EmCD HEK293 Plus Feed Medium is chemically defined medium, free of glutamine, protein, growth factors and any animal-derived components to ensure the safety and stability of the cell culture process.

Cat No.	Packing	Storage Condition	Retest Date
P30310.0001	1L	2-8°C, Protect from light	18 Months
P30310.0005	5L	2-8°C, Protect from light	18 Months
P30310.0010	10L	2-8°C, Protect from light	18 Months
P30306.12kg	12kg	2-8°C, Protect from light	18 Months

Use of product

EmCD HEK293 Plus Feed Medium is mainly used for suspension culture of Expi 293F, HEK293 cells and transient transfection to express antibodies or recombinant proteins.

EmCD HEK293 Plus Feed Medium need to be used with EmCD HEK293 Plus Medium(P30306) and EmCD HEK293 Plus Supplement 1 (L30311) after transfection to improve the protein expression.

Before using EmCD HEK293 Plus Feed Medium, please add glutamine or other additives, such as insulin, according to the requirements of the cell line used.

Preparation for 1L feed A liquid medium

1. Weigh the powder medium, Weigh quantity = Concentration x expected volume of liquid, the concentration of medium: 93.51g/L.
2. Fill the container with injection water (temperature 20-30°C) to 80% of the expected volume of liquid and add the dry powder.
3. Stir until powder is fully dissolved (stir for 15-20 minutes).
4. Adjust pH to 6.60-6.90 with 5mol/L sodium hydroxide solution and continue stirring for 20-30min. The solution is clear now.
5. After the medium was completely dissolved, add injection water to the expected liquid volume and continue to stir for 5 minutes.
6. Immediately use 0.22μm filter membrane with positive pressure for sterilization.
7. The solution should be stored in 2-8°C sealed and avoid from light.

Note

EmCD HEK293 Plus Feed Medium will not be clear until add NaOH solution.

Recommended Protocol for PEI transfection

Before transfection, the cells should be fully adaption in EmCD HEK293 Plus Medium (P30306) . And the subculture was performed with normal seeding density.

1. Incubated condition: 37°C, 5% CO_2, 100rpm (±5) shaker with amplitude 50mm. The conditions could be referred to original process according to actual situation.

Era Scientific(Changzhou)Co., Ltd.
No.7 Hanshan Road
Xinbei District, Changzhou
Jiangsu, China 213125

艾瑞生命科学技术(常州)有限公司
寒山路 7 号
常州新北区
中国江苏 213125

www.eminencebio.com
vip@eminencebio.com
+86 519 81580966
400 928 3628

Day -1: Preparation

2. Cell preparation
   a) Subculture and expand enough cell for transfection.
   b) The day before transfection, subculture the cells (from step (a)) in 2.5-3.0x10^6/mL with fresh EmCD HEK293 Plus Medium. The cell will grow over night (almost 24hours) before transfection.

Day 0: Transfection

3. Prepare the cell for transfection

   Dilute the cell (from step 2) to final density 3.0x10^6/mL with fresh EmCD HEK293 Plus Medium, pre-warmed in incubator at 37℃.

4. Prepare transfection reagent
   a) Place the Plasmid DNA in room temperature for more than 5minutes.
   b) Prepare Transfection complexes as described in table 1 (take 30mL transfection scale for example).

Table 1 Recommended volumes for transfection at 30mL scale

Complexes for Transfection	
Complex A (1.5mL)	
EmCD HEK293 Plus Medium	1.5mL
DNA	30μg
Complex B (1.5mL)	
EmCD HEK293 Plus Medium	1.5mL
Polyethylenimine (PEI, Polysciences, Cat No.:24765)	90μg
Note: Incubate complex A and B at room temperature for 5 minutes prior to mixture	

5. Add complex B to complex A (total 3mL), mix by swirling the tube or pipetting 2-3 times gently.
6. Incubate the mixture at room temperature for 10-20 minutes. Mix the mixture every 5 minutes during the incubation.
7. Transfer the mixture (from step 6) to the cells (27mL from step 3) slowly, swirling the cell gently during the addition.
8. Incubate the cell in 37℃, 5% CO_2, 100rpm (±5) shaker (amplitude 50mm).

Day 1: Supplement addition

9. On the day after transfection (20-24 hours post-transfection), add 1%(v/v) EmCD HEK293 Plus Supplement 1 (L30311) and 3%(v/v) EmCD HEK293 Plus Feed Medium to the cell (from step7). Gently swirling the flask during addition.
10. Maintain glucose concentration above 2g/L during the culture process.

Day 5: Harvest

11. The titer will achieve the peak value for most protein expression at day5-6. Optimal time to harvest protein depend on the cell viability and protein expression.
12. Culture process optimization will be useful for expression.