CD 293 System

Version 3.0

CD 293 system is a chemically defined media platform used for supporting high-density growth and high-efficiency transfection of HEK293 cells in suspension. The system is applicable to expression of recombinant protein and production of viral vectors, such as Adeno-Associated Virus (AAV), Lentivirus (LV), Adenovirus (Adv).

Product	Application	Catalog No.	Form	Package Sizes
CD 293 01	Recombinant protein, Viral vectors	11203-1238	Dry powder	2 L, 10 L, 100 L, Customized
		11203-22052	Liquid	500 mL, 1 L
CD 293 02	Recombinant protein, Viral vectors	11204-1239	Dry powder	2 L, 10 L, 100 L, Customized
		11204-22053	Liquid	500 mL, 1 L
CD 293 03	Viral vectors	11205-1240	Dry powder	2 L, 10 L, 100 L, Customized
		11205-22054	Liquid	500 mL, 1 L
CD 293 06	Recombinant protein, Viral vectors	11207-1652.1	Dry powder	2 L, 10 L, 100 L, Customized
		11207-21018.1	Liquid	500 mL, 1 L
ALLY Feed 100	Feed, used with basal media	99182-1597	Dry powder	2 L, 10 L, 100 L, Customized
		99182-24005	Liquid	500 mL, 1 L

Component Information

Without any components of animal origin, protein, undefined lysates, phenol red and EDTA.

Safety Warning

Read the Material Safety Data Sheets (MSDSs) and following handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Intended Use

For research and further manufacturing use only.

Storage and Stability

Liquid Medium

The liquid medium should be stored at 2–8 °C and protected from light. The product is stable for 12 mouths, when unopened and stored properly. Addition of other supplements may affect storage conditions and shelf life. Do not use any bottle of medium that shows evidence of particular matter or cloudiness.

Powder Medium

The dry powder medium should be stored at 2–8 °C and protected from light. The product is stable for 12 mouths, when unopened and stored properly. This product is very hygroscopic and should be kept in a dry environment away from moisture.

Prepare Liquid Medium from dry powder

11203-1238, 11204-1239, 11205-1240, 11207-1652.1

1. Measure 90% of final volume purified water at room temperature (20–30 °C).
2. Add dry powder to water, mix for a minimum of 30 minutes.
   Note: 21.63 g/L for 11203-1238, 23.33 g/L for 11204-1239, 23.33 g/L for11205-1240, 22.43 g/L for 11207-1652.1
3. Adjust pH to 6.8–7.0 with NaOH solution, mix for 10 minutes.
4. Add 2.20 g/L Sodium Bicarbonate to the solution, mix for 10 minutes.
5. Adjust pH to 7.0–7.4 with NaOH solution or HCl.
6. Dilute to final production volume with ambient purified water, mix for 10 minutes.
7. Sterilize immediately by a 0.22 μm membrane filter.

ALLY Feed 100 (99182-1597)

1. Measure 80% of final volume purified water at room temperature (20–30 °C).
2. Add 189.72 g/L dry powder to the solution, Mix and after dry powder is no longer visible on the surface of the solution, continue mixing for an additional 30 minutes.
3. Adjust the pH to 7.0–7.2 using 5M–10M NaOH. Mix for 60 minutes.

4. Adjust the pH to 7.0–7.2 using 1 M–5 M HCl or NaOH. Mix for 10 minutes. The pH may rise 0.1 to 0.2 after sterile filtration.
5. Adjust the final volume with purified water (density 1.07 g/mL). Mix for 10 minutes.

Culture Conditions

Culture Type: Suspension
Culture Vessels: TPP tubes and shake flasks
Shaking Speed: For TPP tubes, it is recommended 200 rpm @50 mm orbital diameter;
For shake flasks, it is recommended 125–150 rpm @25 mm orbital diameter or 90–120 rpm@50mm orbital diameter.
Culture Temperature: 37 °C
CO2 Concentration: 5%
Relative Humidity: 80% RH

Recover Cells

CD 293 01, CD 293 02, CD 293 03, CD 293 06

1. Rapidly thaw (< 1 minute) frozen cells in a 37 °C water bath.
2. Transfer all cells fluid into a sterile centrifuge tube with 30 mL pre-warmed CD 293 media, centrifuge the cells at 1000 rpm (about × 233 g) for 5 minutes, aspirate and discard the supernatant, transfer cells into a new TPP tube with CD 293 media to an initial cell density of 0.4-0.6 × 10^6 cells/mL.
3. Culture the cells referring to the "Culture Conditions".
4. Passage cells every 72 ± 4 hours, and the passage method should refer to the steps of "Passage Cells and Scale-up".

Passage Cells and Scale-up

CD 293 01, CD 293 02, CD 293 03, CD 293 06

1. Determine the viable cell density and viability after 72 ± 4 hours of recovery.
2. When viable cell density is > 2.0 × 10^6 cells/mL and viability is > 90%, the cells is in the mid-logarithmic phase. Seed at a viable cell density 0.8–1.2 × 10^6 cells/mL to passage. Culture the cells referring to the "Culture Conditions".
3. Ensure a minimum of 3 passages in CD 293 media before transcription.

Adapt Cells to CD 293 Media

CD 293 01, CD 293 02, CD 293 03, CD 293 06

Direct Adaptation

1. Transfer cells grown in other media directly into CD 293 media at 0.8–1.2 × 10^6 cells/mL. Culture the cells referring to the "Culture Conditions".
2. It is recommended to measure the cell density (× 10^6 cells/mL) and viability (%) daily after the first transfer to CD 293 media.
3. After several passages, the viable count should reach at least 4 × 10^6 cells/mL with > 90% viability within 3 days of seeding culture. At this stage, culture is considered to be adapted to CD 293 media.
4. Passage cells refer to the steps of "Passage Cells and Scale-up". The other experiments should be carried out after 3–5 continuous passages.

Sequential Adaptation

1. During sequential adaption of HEK293 cells, seed at a viable cell density of 0.8–1.2 × 10^6 cells/mL.
2. At each subsequent passage, dilute cells with stepwise decreasing the ratio of original medium to CD 293 medium (90 : 10, 75 : 25, 50 : 50, 25 : 75, 0 : 100).
3. If viable cell density is around 3 × 10^6 cells/mL and viability is > 90%, next step can be carried out. 2–3 passages at each step is needed to achieve consistent growth.
4. After several passages in 100% CD 293 media, the viable cell count should reach at 4 × 10^6 cells/mL with > 90% viability within 3 days of seeding culture.
5. Carry out other experiments with HEK293 cells after a minimum of 3–5 passages of successful adaption.

Cryopreservation

CD 293 01, CD 293 02, CD 293 03, CD 293 06

It is recommended to use cells in mid-logarithmic growth phase with > 90% viability for cryopreservation.

1. Prepare the required volume of cryopreservation medium of 90% CD 293 media + 10% DMSO and store at 2–8 °C until use.
2. Determine the viable cell density (× 10^6 cells/mL) and calculate the volume of cryopreservation medium to give a final density of 10.0 × 10^6 cells/mL.
3. Harvest cells by centrifugation at 233 × g for 5 minutes. Resuspend the pellet in the pre-determined volume of 2–8 °C cryopreservation medium.
4. Cell cryopreserved suspension was divided into each cryopreserved tube and immediately transferred to the pre-cooled freezing container, after the programmed cooling (1 °C decrease per minute), transfer them to the liquid nitrogen tank for long-term storage.

Protocols

The following tables illustrate the protocols for utilizing CD 293 System, detailing feeding strategies (including feeding time points and amounts), and culture conditions.

Transient Protein Expression

Feeding Strategies	Basal Medium	Feed	Feeding Timepoints	Feed Amount (% v/v)	Culture Conditions
1	Basal Medium	ALLY Feed 100	24 h and 96 h post plasmid transfection	7% each time	37 °C, 5% CO_2, 80% RH
2			24 h, 72 h, and 120 h post plasmid transfection	5% each time	
3			24 h post plasmid transfection	8–12%	
Note	ALLY Feed 100 contains 80 g/L glucose. It is recommended to add an extra 2 g/L glucose at 96 h post plasmid transfection.				

Production of AAV, AdV and LV

Feeding Strategies	Basal Medium	Feed	Feeding Timepoints	Feed Amount (% v/v)	Culture Conditions
1	Basal Medium	ALLY Feed 100	24 h post plasmid transfection	5%	37 °C, 5% CO_2, 80% RH
2			24 h, 72 h, and 120 h post plasmid transfection	8%	

Related Products

Product	Catalog No.
PBS	99001-014
Glucose Solution (300 g/L)	99024-16023
L-Glutamine (200 mM)	99025-17029