CD 293 System

Version 2.6

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 proteins 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	Basal medium (recombinant proteins, viral vectors)	11203-1238	Dry powder	5 L, 50 L, 100 L, Customized
		11203-22052	Liquid	500 mL, 1000 mL
CD 293 02	Basal medium (recombinant proteins, viral vectors)	11204-1239	Dry powder	5 L, 50 L, 100 L, Customized
		11204-22053	Liquid	500 mL, 1000 mL
CD 293 03	Basal medium (viral vectors)	11205-1240	Dry powder	5 L, 50 L, 100 L, Customized
		11205-22054	Liquid	500 mL, 1000 mL
CD 293 FA	Feed (stable protein production, membrane proteins, viral vectors)	99151-1524	Dry powder	5 L, 50 L, 100 L, Customized
		99151-23060	Liquid	500 mL, 1000 mL
CD 293 FC	Feed (transient protein production)	99151-1327	Dry powder	5 L, 50 L, 100 L, Customized
		99151-23016	Liquid	500 mL, 1000 mL
CD 293 FB	Feed used with CD 293 FA or FC. Don't have to use if the culture duration is less than 5 days.	99035-1242	Dry powder	5 L, 50 L, 100 L, Customized
		99035-23004	Liquid	500 mL, 1000 mL

Component Information

All products don't contain any components of animal origin, proteins, undefined lysates, phenol red, and EDTA. CD 293 01, 02, and 03 contain 4 mM glutamine, 6 g/L glucose, and 1.5 g/L PF68. CD 293 FA and FC contain glutamine and glucose. CD 293 FB doesn't contain glutamine and glucose.

Safety Warning

Read the Material Safety Data Sheets (MSDS) and follow 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 months, 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 24 months, 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

CD 293 01 (11203-1238), CD 293 02 (11204-1239), CD 293 03 (11205-1240)

1. Measure 90% of the 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 for 11205-1240.
3. Adjust pH to 6.8–7.0 with a 1.0N–5.0N 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 a 1.0N–5.0N NaOH solution or HCl.
6. Dilute to the final volume with purified water, mix for 10 minutes.
7. Sterilize the medium immediately using a 0.22 μm membrane filter.

CD 293 FA (99151-1524)

1. Measure 80% of final volume purified water at room temperature (20–30°C).
2. Add 180.10 g/L dry powder, mix for a minimum of 30 minutes.
3. Adjust pH to 6.5–6.9 with a 1.0N–5.0N NaOH solution, mix for 30 minutes.
4. Dilute to the final volume with purified water, mix for 10 minutes.
5. Sterilize the medium immediately using a 0.22 μm membrane filter.

CD 293 FC (99151-1327)

1. Measure 80% of final volume purified water at room temperature (20–30°C).
2. Add 170.00 g/L dry powder, mix for 10 minutes.
3. Adjust pH to 6.5–6.9 using a 1.0N–5.0N HCl solution, mix for 30 minutes.
4. Dilute to the final volume with purified water, mix for 10 minutes.
5. Sterilize the medium immediately using a 0.22 μm membrane filter.

CD 293 FB (99035-1242)

1. Measure 80% of the final volume purified water at room temperature(20–30°C).
2. Add 94.97 g/L dry powder to water, mix for 30 minutes.
3. Adjust pH to 10.8–11.4 with a 1.0N–5.0N NaOH solution, mix for 30 minutes.
4. Dilute to the final volume with purified water, mix for 10 minutes.
5. Sterilize the medium immediately using a 0.22 μm membrane filter.

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
CO_2 Concentration: 5%
Relative Humidity: 80% RH

Recover Cells

CD 293 01, CD 293 02, CD 293 03

1. Rapidly thaw (<1 minute) frozen cells in a 37°C water bath.
2. Transfer all the 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

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 are 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

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 are 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

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 is divided into each cryopreserved tube and immediately transferred to a pre-cooled freezing container, after the programmed cooling (1°C decrease per minute), transfer them to the liquid nitrogen tank for long-term storage.

Process for Transient Protein Expression (PEI)

CD 293 01, CD 293 02, CD 293 FA/FC, CD 293 FB

<table>
<tr><th>Test</th><th>Basal medium</th><th>Feed</th><th>Feeding time points</th><th>Feeding volumes (of working volume)</th><th>Culture conditions</th></tr>
<tr><td>T1</td><td>CD 293 01</td><td rowspan="2">CD 293 FA/FC & CD 293 FB</td><td rowspan="2">24 h, 72 h, 120 h after transfection</td><td rowspan="2">FA/FC: 3%, 3%, 3%.
FB: 0.3%, 0.3%, 0.3%</td><td rowspan="6">37°C, 5% CO_2,
80% RH</td></tr>
<tr><td>T2</td><td>CD 293 02</td></tr>
<tr><td>T4</td><td>CD 293 01</td><td rowspan="2">CD 293 FA/FC & CD 293 FB</td><td rowspan="2">24 h, 72 h, 120 h after transfection</td><td rowspan="2">FA/FC: 5%, 5%, 5%.
FB: 0.5%, 0.5%, 0.5%</td></tr>
<tr><td>T5</td><td>CD 293 02</td></tr>
<tr><td>T7</td><td>CD 293 01</td><td rowspan="2">CD 293 FA/FC</td><td rowspan="2">24 h after transfection</td><td rowspan="2">FA/FC: 10–15%</td></tr>
<tr><td>T8</td><td>CD 293 02</td></tr>
<tr><td>Note</td><td colspan="5">(1) Supplement glucose to 5 g/L at every feeding time point.
(2) Supplement 4 mM of L-glutamine at every feeding time point (non-GS system).</td></tr>
</table>

Process for Production of AAV, AdV, and LV

CD 293 03, CD 293 FA, CD 293 FB

<table>
<tr><th>Test</th><th>Basal medium</th><th>Feed</th><th>Feeding time points</th><th>Feeding volumes (of working volume)</th><th>Culture conditions</th></tr>
<tr><td>T1</td><td>CD 293 03</td><td>CD 293 FA & CD 293 FB</td><td>24 h, 72 h, 120 h after transfection or infection</td><td>FA: 3%, 3%, 3%.
FB: 0.3%, 0.3%, 0.3%</td><td rowspan="3">37°C, 5% CO_2,
80% RH</td></tr>
<tr><td>T2</td><td>CD 293 03</td><td>CD 293 FA & CD 293 FB</td><td>24 h, 72 h, 120 h after transfection or infection</td><td>FA: 5%, 5%, 5%.
FB: 0.5%, 0.5%, 0.5%</td></tr>
<tr><td>T3</td><td>CD 293 03</td><td>CD 293 FA</td><td>24 h after transfection or infection</td><td>FA: 10–15%</td></tr>
<tr><td>Note</td><td colspan="5">(1) Supplement glucose to 5 g/L at every feeding time point.
(2) Supplement 4 mM L-glutamine at every feeding time point (non-GS system).</td></tr>
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Related Products

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