SAFC®
Pharma & Biopharma Raw Material Solutions

Product information sheet

Cellvento® 4HEK Medium

For Producing High AAV Titers in Multiple HEK293 Cell Lines

Product Description

Cellvento® 4HEK Medium is a chemically defined medium optimized for adeno-associated virus (AAV) production in multiple human embryonic kidney (HEK) 293 lineages. Cellvento® 4HEK Medium has been streamlined and cell culture media components incorporated to increase media stability and reduce protein oxidation. The formulation is animal component free.

Application

Cellvento® 4HEK Medium can be used as amplification and production medium for gene therapy applications. This medium enables low doubling time and high viable cell densities with multiple HEK293 cell lines. Cellvento® 4HEK Medium is compatible with commonly used transfection reagents like PEI, supports AAV production, and facilitates high titers of full capsids in various HEK293 lineages. This easy-to-use medium can be used for all stages like growth, transfection, and production.

This product is intended for research or further manufacturing but not for human or therapeutic use.

Media preparation

Liquid medium must be supplemented with 6–10 mM L-Glutamine.

Dry powder medium must be supplemented with 2.5 g/L Sodium Bicarbonate. Once hydrated, medium must be supplemented with 6-10 mM L-Glutamine.

Storage

Liquid and dry powder media must be stored at 2-8 °C and protected from light.

Shelf life

12 months for liquid and dry powder media.
Do not use after expiration date.

Reconstitution method to prepare 1 L Cellvento® 4HEK Medium from dry powder medium

1. Add 800 mL of Milli-Q® or similar culture grade water in an appropriately sized container.
2. Add 20.76 g of Cellvento® 4HEK Medium and stir for 20 minutes.
3. Add 1.4 mL of 5N NaOH to adjust pH to >6.5.
4. Add 2.5 g of sodium bicarbonate.
5. Adjust pH to 7.2-7.3 (using 5N HCl/5N NaOH as needed).
6. Add cell culture grade water to reach a final volume of 1,000 mL.
7. Sterile filter using a sterilizing-grade filter (≤ 0.22 µm).

Note: This medium does NOT contain L-Glutamine. Aseptic supplementation to 6-10 mM required prior to use.

Initiating Cultures

1. Aseptically add 19 mL of Cellvento® 4HEK Medium (with 6–10 mM L-Glutamine) to a 125 mL disposable, vented, non-baffled shake flask in a biological safety cabinet.
2. The medium can be used directly from the refrigerator without prewarming to room temperature.
3. Quickly thaw a cryovial of frozen HEK293 cells by placing the vial into a 37 °C water/bead bath or similar method until just a sliver of ice remains.
4. In the biological safety cabinet, use a disposable pipette to immediately transfer, by careful pipetting, the contents of the vial to the shake flask (vented cap) containing 19 mL Cellvento® 4HEK Medium that was prepared in step 1. Swirl culture flask gently to mix.
5. Count the cells to ensure viability is >85%.
6. Incubate cells at 37 °C, 5% CO_2, 80% relative humidity, with an agitation of 130–140 rpm on a 25 mm throw platform.
7. Count cells 72 hours post-thaw to culture.

Recommended seeding densities for routine cell maintenance:

Passage Timing	Recommended Seeding Density
For 3-day cultures:	0.3–0.5 x 10^6 viable cells/mL
For 4-day cultures:	0.2–0.3 x 10^6 viable cells/mL

Recommended volumes for routine cell culture maintenance in vented shake flasks (baffled or non-baffled).

Flask Size	Culture Volume	Parameter
125 mL	25–30 mL	155 ±5 rpm (19 mm shaking diameter)
250 mL	55–65 mL	135 ±5 rpm (25 mm shaking diameter)
500 mL	145–155 mL	95 ±5 rpm (50 mm shaking diameter)
1 L	280–300 mL	

Note: Baffled flasks can be used to reduce clumping or improve growth for some HEK293 cell lines.

1. Use the viable cell density to calculate the volume of cell suspension required to seed a new shake flask according to the recommended seeding densities and the recommended culture volume.
2. Transfer the calculated volume of cells to fresh Cellvento® 4HEK Medium (supplemented with 6–10 mM L-Glutamine) in a shake flask.
3. Incubate at 37 °C, 5% CO_2, 80% relative humidity, with an agitation of 130–140 rpm on a 25 mm throw platform for 3–4 days (until the cell cultures reach a density of 3.5–5 x10^6 viable cells/mL).
4. Repeat steps 1–3 to maintain or expand cells.

Cryopreservation

1. Prepare the desired quantity of cells (10 x 10^6 viable cells/mL), harvesting in mid-logarithmic phase of growth with viability >95%.
2. Prepare freezing medium, which is composed of Cellvento® 4HEK Medium (supplemented with 6–10 mM L-Glutamine) and 7.5% DMSO (v/v). Sterile filter freezing medium and keep at 4 °C.
3. Harvest cells by centrifugation at 200 x g for 3 minutes. Remove supernatant.
4. Resuspend cells in the freezing medium prepared in step 2.
5. Rapidly transfer 1 mL of the cell suspension to a 2 mL cryovial and cap tightly.
6. Place vials immediately in cryovial freezing container prechilled at 4 °C and freeze cells at a rate of –1 °C to –3 °C per minute.
7. For long-term storage (>1 month), transfer the vials to liquid nitrogen (vapor phase).

Ordering information

Product	Cat. No.
Cellvento® 4HEK Liquid Medium	
CCellvento® 4HEK 1000 mL bottle	1251931000
Cellvento® 4HEK Dry Powder Medium (DPM)	
Cellvento® 4HEK DPM for 5 L	1251950005
Cellvento® 4HEK DPM for 10 L	1251950010
Cellvento® 4HEK DPM for 25 L	1251950025
Cellvento® 4HEK DPM for 50 L	1251950050
Cellvento® 4HEK DPM for 100 L	1251950100
Related Products	
L-Glutamine	G7513
Sodium Bicarbonate	S5761

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