Product Information

HEK Pro (A) Medium

Serum-Free, Chemically Defined Medium, w/o L-Glutamine

HEK Pro (A) cell culture medium has been formulated to support high growth and transfection in Human embryonic kidney 293 (HEK293) suspension cell lines. HEK Pro (A) is a chemically defined (CD), serum-free (SF), animal origin-free (AOF) medium that contains no protein, hydrolysates, or components of unknown composition. The medium has been optimized to promote high yields of adenovirus and recombinant protein production. The medium is formulated without L-glutamine and without phenol red to minimize estrogen-like effects of phenol red.

Specification Table

Classification	Serum	Animal Origin	Protein	Endotoxin	Antibiotics	Phenol Red	Glutamine
Chemically Defined	Free	Free	Free	< 5 EU/mL	No	No	No

Storage and Shelf-life

HEK Pro (A) medium is highly hygroscopic powder and should be stored in dry at 2°C to 8°C protected from light and moisture. The entire contents of each package should be used immediately after opening. Please refer to the label for the expiry date.

Culture conditions

Media: HEK Pro (A)
Cell line: HEK293 cells
Culture type: Suspension
Temperature range: 36°C to 38°C
Incubator atmosphere: Humidified atmosphere of 5-8% CO2 in air**.**
Culture vessels: Shake flasks, spinner bottles, and bioreactor.

Reconstitute HEK Pro (A) medium

1. Take 90 % of the final volume of cell culture grade water at an ambient temperature into an appropriately sized mixing vessel.
2. Add the dry powder medium (22.03 g/L) slowly to the water and mix gently for 30-45 minutes.
3. Add 2.2 g of sodium bicarbonate per liter of the final volume of the medium and stir until dissolved (about 20 minutes).
4. Add cell culture grade water to achieve the appropriate final volume and mix well.
5. Measure pH and Osmolality
6. Sterilize the medium immediately by using 0.22 µm filter. Aliquot the sterile solution under aseptic conditions to avoid contamination, and store the medium at 2–8 °C, protected from light for 6 months

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Complete medium preparation for use

1. Supplement HEK Pro (A) medium with L-glutamine at 4-8 mM final concentration prior to us. Recommended concentration is 4mM.
2. Glucose supplementation may be required for terminal batch cultures and should be determined empirically.
3. Anti-clumping agent can be added to reduce cell aggregation. Concentration of anti-clumping agent can be determined experimentally for individual cultures or can be supplemented based on suppliers recommendation.

NOTE: It is important to remove anti-clumping agent 1-2 subcultures before transfection as its components can adversely affect transfection efficiency.

Recover frozen cells

1. Rapidly thaw (about 1.5 minute) frozen cells in a 37°C water bath.
2. Transfer the contents of the cryovial into a 125-mL shake flask containing 30 mL

complete HEK Pro (A) Medium.

3. Incubate at 37°C in a humidified atmosphere of 5-8% CO2 in air with shaking speed of 125–135 rpm.
4. Maintain a cell density of 0.5 × 10^6–1.5 × 10^6 viable cells/mL for the first two passages following recovery; thereafter, return to your normal maintenance schedule.

Subculture cells

1. Determine viable cell density using an automated cell counter or manual methods. Ensure that the cell density is ≥1 × 10^6 viable cells/mL, viability is ≥90%, and cells are in the mid-logarithmic phase prior to subculturing.
2. Calculate the volume of cell culture and medium necessary to seed a flask at 3 × 10^5 viable cells/mL in a total volume of 30 mL fresh HEK PRO (A) medium per 125-mL shake flask.
   NOTE: If cell density does not reach 1 × 10^6 viable cells/mL within 5 days of recovery, centrifuge cells at 100 × g for 5 minutes and resuspend the cell pellet in 20–30 mL of fresh complete HEK Pro (A) medium.
5. Incubate at 37°C in a humidified atmosphere of 5-8% CO2 in air with shaking speed of 125–135 rpm.
6. Subculture cells by seeding at density of 3 × 10^5 viable cells/mL every 2–3 days with fresh HEK PRO (A) medium.

NOTE: VV Pro (A) cultures may grow as cell clusters of 5-10 cells. Prior to the routine subculture, allow large cell clumps to settle to the bottom of the culture vessel, then carefully remove the required culture of suspended cells and dilute it with the fresh medium in a new vessels. Vortexing for short durations may be required for several subcultures until single cell growth is observed in subsequent subcultures. Maintaining cells at lower densities between 0.2-2.5 x 10^6 viable cells/mL by frequent subcultures (3 times every week) can help improve transfection process.

Adaptation of HEK293 cells to HEK Pro (A) Medium

We recommend adapting HEK293 cells to HEK Pro (A) medium using sequential adaptation. However, some HEK cell lines will adapt directly from other medium, especially those which are being maintained in other serum-free medium. It is critical that cell viability be ≥90% and the growth is in mid-logarithmic phase prior to initiating adaptation procedures.

Sequential adaptation

1. Expand the culture grown in conventional 5–10% serum-supplemented medium or other serum-free medium until cells are in logarithmic phase having viability greater than 90%.
2. Dilute cells by seeding at 4 × 10^5– 5 × 10^5 viable cells/mL in 1:3 (25:75) ratio of complete HEK PRO (A) medium to the original medium.
3. Subculture cells at 4 × 10^5– 5 × 10^5 viable cells/mL in the same medium when viable cell density reaches ≥1 × 10^6 cells/mL. Once consistent cell growth with high viability (>90%) is achieved, subculture cells into 1:1 (50:50) ratio of complete HEK PRO (A) medium to the original medium.
4. Repeat step 3 by stepwise reducing the original medium [3:1 (75:25) and 9:1 (90:10)] followed by 100% HEK PRO (A) medium. Multiple passages at each step may be needed.
5. After several passages in 100% HEK PRO (A) medium, the viable cell count should reach at least 2 × 10^6 cells/mL with ≥85% viability within 3–5 days of seeding culture. At this stage, the culture is considered to be adapted to HEK Pro (A) medium.

NOTE

- Make a frozen stock of the cells in the original medium prior to adaptation.
- Keep a culture going of the cells in each prior condition when starting the next level of adaptation as a fall-back if the cells do not survive in the next passage.
- Make 2-3 vials during each step of sequential adaptation as a back-up.

Direct adaptation

1. For direct adaptation of HEK cells grown in

other serum-free medium into HEK Pro (A) medium, dilute cells into 100% HEK Pro (A) medium by seeding at 4–5 × 10^5 viable cells/mL when subculturing.

2. Continue to subculture cells at 4–5 × 10^5 viable cells/mL (every 3–4 days) until consistent growth is achieved. Once cell growth has been demonstrated, the seeding density may be reduced to 3 × 10^5 viable cells/mL during the final stages of adaptation.
3. After several passages in HEK Pro (A) medium, the viable cell count should reach at least 2 × 10^6 cells/mL with ≥85% viability within 3–5 days of seeding culture. At this stage, the culture is considered to be adapted to HEK PRO (A) medium

NOTE: If suboptimal performance is achieved using the direct adaptation method, use the sequential adaptation method.

Cryopreservation

1. Prepare the desired quantity of cells, harvesting in mid-log phase of growth having viability >90%.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of >1 × 10^7 cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% HEK Pro (A) medium (50:50 ratio of fresh to conditioned medium) + 7.5% DMSO and store at 4°C until use.
   NOTE: Prepare cryopreservation medium on the day of use.
4. Harvest cells by centrifugation at 100 × g for 5–10 minutes. Resuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications.
6. Achieve cryopreservation in an automated controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen (vapor phase) storage at –200°C to –125°C.

NOTE: Check viability of cryopreserved cells 24 hours after storage of vials in liquid nitrogen (see "Recover frozen cells" section).

Precautions and Disclaimer

This product is for research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices. We provide information to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations should be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information does not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

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Help Needed?

For any further questions regarding this product, please contact us at info@sarvoshadhi.com.