HEK 293 Medium, with Glutamine

Cat. No.: H1492269 | Pack size: 500 mL | Storage: Store at 2-8°C; protected from light

Overview

This product is a chemically defined medium, free of serum, proteins, growth factors, animal-derived components, and hydrolysates. It is suitable for the suspension culture of HEK 293 cells to achieve rapid cell proliferation, high-density culture, and efficient transient transfection.

Main Components

- Composed of water, glucose, amino acids, vitamins, inorganic salts, trace elements, sodium bicarbonate, and a pH buffer system.
- Free of serum, proteins/growth factors, animal-derived components, hydrolysates, HT, and phenol red; chemically defined.
- Contains 4 mM Glutamine.

This product is suitable for the culture of HEK 293 cells in suspension.

Instructions for Use

1. Basal Culture Conditions

- Culture Temperature: 37.0 ± 0.5°C (can be adjusted according to the cell culture process).
- CO_2 Concentration: 5~8%.
- Humidity: ≥80%.
- Shaker Speed: 110~150 rpm (with an amplitude of 50 mm).

2. Cell Thawing and Recovery

2.1 Pre-warm the medium completely at 37°C, protected from light. Transfer 10 mL of the pre-warmed medium into a 50 mL centrifuge tube and set aside.

2.2 Quickly thaw the frozen cell vial in a 37°C water bath (for less than 2 min).

2.3 Transfer the thawed cell suspension to the prepared centrifuge tube containing the medium. Centrifuge at 300 g for 3 min and discard the supernatant. Resuspend the cell pellet in 10 mL of pre-warmed medium. Take a sample to count cells and determine cell density and viability.

2.4 Transfer the cell suspension to a shake flask/tube. Dilute to the desired seeding density with pre-warmed medium and culture according to the basal culture conditions.

3. Cell Passaging (Subculture)

3.1 Pre-warm the medium completely at 37°C, protected from light.

3.2 Select cells in the logarithmic growth phase, or with a viable cell density (VCD) ≥ 1.5 × 10^6 cells/mL and viability ≥ 90%. Subculture at the desired seeding density according to experimental requirements.

3.3 Recommended cell seeding density: 0.3~0.7 × 10^6 cells/mL.

3.4 After routine culture for 3-5 days, perform the next subculture.

4. Cell Cryopreservation

4.1 Prepare Freezing Medium: Use this medium as the base and add DMSO to a final concentration of 10% (v/v). Pre-cool the mixture at 2-8℃ until ready for use.

4.2 Select cells in the logarithmic growth phase with viability ≥ 90%. Recommended final cryopreservation density: 1.0~3.0 × 10^7 cells/mL.

4.3 Calculate the required cell suspension volume based on the desired cryopreservation density. Centrifuge the required volume of cells at 300 g for 5 min to pellet them. Discard the supernatant and resuspend the cell pellet gently in the pre-cooled freezing medium.

4.4 Immediately aliquot the cell suspension into pre-chilled cryogenic vials. Freeze following a standard slow-freezing program (cooling rate of -1℃/min) using a controlled-rate freezing apparatus until reaching -80℃. Then transfer the vials to a -80℃ freezer for overnight storage.

4.5 For long-term storage, cells must be transferred to liquid nitrogen.

5. Cell Adaptation (Acclimation)

This product supports both direct and gradual adaptation methods. The criteria for successful adaptation are: after 3-4 days of culture using 100% of this medium, the cell density should reach ≥ 3.0 × 10^6 cells/mL with viability ≥ 90%.

5.1 Direct Adaptation: Inoculate cells directly into this medium at a seeding density within the recommended range for passaging (0.3~0.7 × 10^6 cells/mL). Cells should achieve stable growth after 3-6 consecutive passages.

5.2 Gradual Adaptation: If direct adaptation is not optimal, select low-passage, logarithmically growing cells. Replace the medium gradually according to the ratios below. Maintain a seeding density of 0.4~0.6 × 10^6 cells/mL for each passage during the adaptation process. Proceed to the next gradient only after the cells have achieved VCD ≥ 3 × 10^6 cells/mL and VIA ≥ 90% for at least two consecutive passages.

Gradient	This Medium : Original Medium
0	0 : 100
1	25 : 75
2	50 : 50
3	75 : 25
4	100 : 0

6. Transient Transfection

These instructions provide general transfection conditions. Optimal parameters should be determined based on the specific experimental setup and can be optimized using Design of Experiments (DOE). The following procedure uses a 10 mL transfection volume, cell density of (3.0 ± 0.5) × 10^6 cells/mL, 10 µg DNA, and a DNA:PEI ratio of 1:4 as an example.

General Transfection Parameter Recommendations:

Parameter	Recommended Range
Cell Density (VCD)	2.0 ~ 4.0 × 10^6 cells/mL
DNA Amount	1.0 ~ 2.0 mg/L
DNA:PEI Ratio (w/w)	1:3~1:6

Transfection Procedure

6.1 Expand HEK 293 cells using this medium until the cell density reaches ≥ (3.0 ± 0.5) × 10^6 cells/mL with viability ≥ 95%.

6.2 Day before transfection: Calculate the required cell suspension volume based on a seeding density of 1.5 × 10^6 cells/mL. Centrifuge the calculated volume of cells at 300 g for 5 min. Discard the entire supernatant. Resuspend the cell pellet in fresh, pre-warmed medium. Culture according to the basal conditions.

6.3 Day of transfection: Take a sample and count cells. Confirm that the cell density is (3.0 ± 0.5) × 10^6 cells/mL and viability is ≥ 95% before proceeding with transfection. If the density is too high, dilute the cells to the target density using fresh, pre-warmed medium (discard excess cells; do not use them for routine passaging).

6.4 Reagent Dilution: Dilute 10 µg of DNA in 0.45 mL of this medium. Mix gently by pipetting 5-10 times. Incubate at room temperature for 5-10 min. Simultaneously, dilute 40 µg of PEI in 0.45 mL of this medium. Mix using the same method.

6.5 Complex Formation: Slowly add the diluted PEI solution to the diluted DNA solution. Mix gently by pipetting 5-10 times. Incubate at room temperature for 10-15 min.

6.6 Transfection: Slowly add the DNA-PEI complex dropwise to the cell suspension. Gently swirl or shake the culture vessel while adding to ensure even distribution of the complexes.

6.7 Culture: Return the culture vessel to the shaker and continue incubation under the basal culture conditions.

6.8 Monitoring: Starting from day 2 post-transfection, take samples daily for cell counting and glucose measurement. Maintain glucose concentration around 4 g/L by supplementing if necessary.

6.9 Harvesting: Protein expression typically peaks 5-7 days post-transfection. It is recommended to stop the culture and harvest the supernatant 6-9 days post-transfection, or when cell viability drops below 60%.

Notes

- This product has been sterilized by 0.22/0.45 μm filtration prior to shipping. Re-filtering is not recommended.
- The product contains 4 mM Glutamine and can be used directly. Additional supplementation may be applied based on the specific requirements of the HEK 293 cell line used.
- The product does not contain ACA and can be used directly. Additional supplementation may be applied based on specific requirements.
- If slight turbidity or precipitation appears after long-term storage at 2-8℃, after ruling out contamination, this is likely due to temperature fluctuations within the refrigerator. To remedy this, warm the bottle to room temperature or in a 37℃ water bath to re-dissolve the components. The medium should return to a clear state and can be used normally.
- Avoid repeatedly warming up the medium during use. It is recommended to use the entire contents within 30 days after opening the bottle.
- During the cell adaptation process, cell performance may be suboptimal for the first 3 passages. Cell characteristics are expected to gradually improve starting from the 4th passage onwards.

Specifications

Attribute	Value
Synonyms	293 Cell Culture Medium (Serum-free) | Serum-Free Chemically Defined HEK293 Suspension Culture Medium with 4 mM L-Glutamine
Specifications & Purity	BioReagent, for cell culture, sterile-filtered
Stability And Storage	Store at 2-8℃ long term (12 months). Store in the dark.
Storage Conditions	Store at 2-8℃, Protected from light
Shipped In	Wet ice. This product requires cold chain shipping. Ground and other economy services are not available.
Grade	BioReagent, for cell culture, sterile-filtered
Sensitivity	Light-sensitive

Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, our regional teams are ready to assist. Please contact the office for your region; for general inquiries, the North American office is the corporate primary.

NORTH AMERICAN SALES, SUPPORT & GENERAL INQUIRIES	EU SALES, LOGISTICS & LOCALIZED SUPPORT

Aladdin Scientific Corporation
14078 Meridian Parkway, Riverside, CA 92518, USA
Phone: 1-833-552-7181
Sales: sales@aladdinsci.com
Customer Service: custserv@aladdinsci.com

Aladdin Biochem Deutschland GmbH
Westring 2, 33142 Büren, Germany
Phone: +02951 9383958
Support: support.eu@aladdinsci.com

Limitations & Disclaimer

For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, or household use.

This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals.

Where any kit component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS) takes precedence over this document for handling, storage, transportation, disposal, and emergency procedures.

Performance depends on sample type, sample condition, handling, and operator technique. Users are responsible for validating the product for their specific application.

Aladdin product labels, SDS, COA, and approved specifications take precedence over this document. If product formulation, label, SDS, storage conditions, pack size, or quality specifications change, this document must be revalidated before use.