

TheraPEAK 293-GT[®] Media System

Instructions for use

I. Introduction

The TheraPEAK 293-GT[®] Media System is a high-performance cell culture media and supplement system optimized to support adeno-associated viral (AAV) vector production in Human Embryonic Kidney (HEK293) suspension cells.

TheraPEAK 293-GT[®] Medium is a protein-free, chemically-defined medium specifically designed to support the growth and transfection of HEK293 suspension cells. TheraPEAK 293-GT[®] Medium is pre-formulated with 4 mM L-alanyl-L-glutamine to promote consistent cell culture performance.

TheraPEAK 293-GT[®] AAV Supplement is a protein-free, chemically-defined supplement formulated to enhance transient transfection-based AAV production. To preserve optimal performance, the supplement should be kept separate from the TheraPEAK 293-GT[®] Medium and added to the culture no more than one hour prior to transfection.

These instructions are intended for performing transfection(s) in a 125 mL shake flask with a working volume of 30 mL cell culture. It is recommended to begin at this scale to establish process performance. The culture volume may subsequently be scaled up as needed to meet specific user requirements.

II. Storage

TheraPEAK 293-GT[®] Media System should be stored at 2–8 °C and protected from light.

III. Instructions for use

Compatibility

TheraPEAK 293-GT[®] Media System has demonstrated compatibility with a variety of commercially available HEK293 suspension cell lines, plasmids and transfection reagents, enabling flexibility across diverse AAV production workflows. Users may select and utilize HEK293 suspension cell lines, plasmids and transfection reagents according to their established protocols and process requirements.

Equipment requirements and operating conditions

Incubator: 37 °C, relative humidity ≥ 80%, 5% CO₂.

Orbital Shaker: 120 ± 5 rpm using a shaker with a 19–25 mm orbit diameter

Water Bath: 37 ± 2 °C

BSC: A Class II Biosafety Cabinet

Material and reagent requirements

Cell Line: HEK293 suspension cell line, as specified in user's protocol.

Medium: Lonza's TheraPEAK 293-GT[®] Medium
Supplement: Lonza's TheraPEAK 293-GT[®] AAV Supplement

Plasmid DNA: Plasmid DNA should be selected based on the requirements of the intended application and production process. The use of a triple-plasmid system is recommended, consisting of:

- A transfer plasmid containing the gene of interest,
- A Rep/Cap plasmid, and
- A Helper plasmid.

For optimal transfection performance, plasmid DNAs should meet the following quality specifications:

- Verified sequence accuracy,
- 260/280 ratio between 1.8 and 2.0,
- Endotoxin levels of ≤ 0.01 EU/ μ g, and
- $90\% \pm 10\%$ supercoiled DNA content.

The use of plasmid DNA that does not meet these quality criteria may result in reduced transfection efficiency and AAV production performance. Plasmid preparations are assumed to be formulated in 1X TE buffer or double distilled (dd) H₂O.

Transfection reagents: Transfection reagents are not supplied by Lonza and should be selected by the user based on the specific cell line, plasmid construct, and transfection protocol. Preparation of transfection complexes should be performed according to the manufacturer's instructions.

General guidelines for HEK293 cell culture in shake flasks

Cells may be thawed and resuspended directly in TheraPEAK 293-GT[®] Medium. However, for optimal growth and productivity, certain HEK293 suspension cell lines may benefit from an adaptation step, in which cells are thawed and cultured in their native medium for 3–5 passages prior to being subcultured into TheraPEAK 293-GT[®] Medium. Users should refer to the cell line manufacturer's recommendations for any specific thawing and recovery procedures to ensure optimal cell performance.

Recommended procedure for revival of HEK293 cells in TheraPEAK 293-GT[®] Medium

1. Warm 30 mL of TheraPEAK 293-GT[®] Medium at 37 °C for approximately 15 min.
2. Thaw frozen vials of HEK293 cells in a 37 ± 2 °C water bath according to user's or cell line manufacturer thawing procedure.
3. Aseptically transfer the contents of the HEK293 cell vial into a 125 mL shake flask containing 30 mL of pre-warmed TheraPEAK 293-GT[®] Medium using a sterile pipette.

4. Measure viable cell density and cell viability using the user's established cell counting method. To maximize recovery and growth following thaw, an initial viable cell density of at least 0.3×10^6 cells/mL is recommended.
5. Gently swirl the shake flask to ensure an even cell distribution and place it on an incubated orbital shaker set to the following conditions:
 - Temperature: 37 °C
 - Relative Humidity: $\geq 80\%$
 - CO₂: 5%
 - Shaking Speed: 120 ± 5 rpm
 - Orbit Diameter: 19–25 mm

Recommended procedure for subculturing HEK293 cells in TheraPEAK 293-GT[®] Medium

1. Perform the initial passage 96 ± 2 hours post-thaw or when the viable cell density reaches $3.0\text{--}4.0 \times 10^6$ cells/mL, whichever occurs first.
2. Perform subsequent passages every 3–4 days to maintain optimal cell growth and viability.
3. Measure viable cell density and cell viability using the user's established cell counting method.
4. Dilute culture with fresh TheraPEAK 293-GT[®] Medium and seed a 125 mL shake flask at an initial viable cell density of $0.5\text{--}0.7 \times 10^6$ cells/mL
5. Maintain cells in TheraPEAK 293-GT[®] Medium for a minimum of three passages prior to initiating AAV production to ensure culture adaptation and consistent performance.

Table 1 summarizes the recommended target values and operating ranges for HEK293 cell revival and subculturing using TheraPEAK 293-GT[®] Medium.

Table 1. Recommended parameters and target Values for HEK293 cell revival and subculturing

Parameter	Recommended target
Medium warming temperature	37 °C
Medium warming time	15 min
Revival seeding viable cell density	$\geq 0.3 \times 10^6$ cells/mL
Cell culture incubation temperature	37 °C
Cell culture incubation relative humidity	$\geq 80\%$
Cell culture incubation CO ₂	5%
Culture vessel	125 mL shake flask
Culture volume	30 mL
Shaking speed	120 $\pm$ 5 rpm
Orbit diameter	19–25 mm
Subculture seeding viable cell density	$0.5\text{--}0.7 \times 10^6$ cells/mL
Subculture duration	3–4 days

Recommended procedure for AAV production using TheraPEAK 293-GT® Media System

1. Three days prior to transfection, seed the HEK293 cells in TheraPEAK 293-GT® Medium at a viable cell density of $0.5\text{--}0.8 \times 10^6$ cells/mL.
2. On the day of transfection, dilute cells with fresh TheraPEAK 293-GT® Medium to achieve the desired cell density for transfection (per transfection reagent protocol). A target viable cell density of $2.5\text{--}3.5 \times 10^6$ cells/mL is typically recommended.
3. Within 1 hour prior to transfection, add TheraPEAK 293-GT® AAV Supplement to the culture at a ratio of 1 mL supplement per 30 mL cell culture. Gently mix to ensure uniform distribution.
4. Prepare transfection complexes in accordance with the manufacturer's instructions for the selected transfection reagents.
5. Perform transfection according to the user's established protocol and the recommendations provided by the transfection reagent manufacturer.

Table 2 summarizes the recommended target values and operating ranges for transfection using TheraPEAK 293-GT® Media System. As transfection performance may vary depending on the cell line, plasmid system, and transfection reagent employed, optimization of process parameters may be needed to achieve optimal AAV production.

Table 2. Recommended parameters and target values for AAV production using the TheraPEAK 293-GT® Media System

Parameter	Recommended target
Number of passages prior to transfection	3 to 20
Transfection subculture seeding density	$0.5\text{--}0.8 \times 10^6$ cells/mL
Transfection subculture cell viability	$\geq 95\%$
Culture vessel	125 mL shake flask
Culture volume	30 mL
TheraPEAK 293-GT® Supplement addition ratio	1 mL per 30 mL of cell culture
TheraPEAK 293-GT® Supplement addition time	Within 1 hour prior to transfection
Shaking speed	120 $\pm$ 5 rpm
Cell culture incubation CO ₂	5 %
Temperature	37 °C
Transfection cell density	$2.5\text{--}3.5 \times 10^6$ cells/mL or as recommended by the transfection reagent manufacturer*
Subculture duration prior to transfection	72 $\pm$ 2 hours
Production duration	72 $\pm$ 2 hours

* Further optimization of transfection cell density may be beneficial for specific cell lines, transfection reagents, and process conditions.

Harvest procedures

AAV harvest and cell lysis should be performed according to the user's established procedures.

Product list		
Reagent	Catalog number	Vendor
TheraPEAK 293-GT® Medium	BP18-971	Lonza Bioscience
TheraPEAK 293-GT® Supplement		

Product use statement

All TheraPEAK® Products are produced according to applicable GMP standards and follow the USP/EP guidance for cell and gene therapy raw materials. Lonza Group Ltd. and its affiliates (collectively and individually, "Lonza") make efforts to include accurate and up-to-date information. However, Lonza makes no representations or warranties, express or implied, including as to accuracy or completeness of information. This product is not for human or animal *in vivo* or diagnostic use, including use as a diluent or as an excipient. This product is suitable for GMP manufacturing. All trademarks belong to Lonza Group Ltd. and its affiliates (collectively and individually, "Lonza"), and are registered in the USA, EU and/or CH, or used in common law, or belong to third-party owners and are used for only informational purposes. All third-party copyrights have been reproduced with permission from their owners. User assumes all risks of product use and handling. Lonza makes efforts to include accurate and up-to-date information. However, Lonza makes no representations or warranties, express or implied, including as to the accuracy or completeness of information, or the use or handling of these products. The user is responsible for determining if the products supplied by Lonza and the information and recommendations given by Lonza are (i) suitable for intended process or purpose, (ii) are in compliance with environmental, health and safety regulations, and other regulations in the regions and countries where they are purchased, offered for sale, marketed, sold, and used and (iii) will not infringe any third party's intellectual property rights. The user bears the sole responsibility for determining the existence of any such third-party rights, as well as obtaining any necessary licenses and approvals. For more information, including regarding legal disclaimers, Lonza's intellectual property rights, and how Lonza collects, uses and protects personal information: www.lonza.com/legal, <https://www.lonza.com/about-us/strategy/intellectual-property> and www.lonza.com/privacy.

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