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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 (HEK 293) suspension cells.

TheraPEAK 293-GT® Medium is a protein free, chemically-defined medium designed for growth and transfection of HEK 293 suspension cells. TheraPEAK 293-GT® Medium is pre-formulated with 4 mM L-alanyl-L-glutamine.

TheraPEAK 293-GT® AAV Supplement is a protein-free and chemically-defined supplement for enhancing transfection-based AAV production. The supplement should be kept separate from the TheraPEAK 293-GT® Medium and added to the medium up to one hour prior to transfection.

II. Storage

TheraPEAK 293-GT® Media System should be stored at 2 to 8 °C and protected from light. Do not subject either product to extreme temperature fluctuations.

III. Instructions for use

Compatibility

TheraPEAK 293-GT® Media System is designed to support HEK 293 suspension cells and is known to be compatible with various commercially available HEK 293 suspension cell lines and transfection reagents.

Culture conditions

Incubator setting: 37 °C, relative humidity ≥ 80%, 5% CO_2.

Shaker setting: 120 ± 5 RPM for an orbital 19 – 25 mm platform

General guidelines for HEK 293 cell culture in shake flasks

Users should follow aseptic cell culture techniques. Cells may be thawed and resuspended directly in TheraPEAK 293-GT® Medium. For optimal performance, certain cell lines may benefit from a recovery process, in which cells are thawed and cultured in their native medium for 3 – 5 passages before being subcultured into TheraPEAK 293-GT® Medium. See manufacturers' instructions for best practices.

Revival of HEK 293 Cells in TheraPEAK 293-GT® Medium

1. Thaw frozen vials of HEK 293 cells in a 37 ± 2 °C water bath according to standard thawing protocols.

Note: To ensure no water from the waterbath comes into contact with the neck of the vial, consider thawing in a sealed plastic bag as a means of preventing microbial contamination.

2. Centrifuge the cells at 200 xg for 7 – 10 minutes and remove cryopreservation medium from the cell pellet via aspiration.
3. Resuspend the cells pellets with 30 mL of prewarmed TheraPEAK 293-GT® Medium by pipetting up and down 3 – 5 times. Transfer the resuspended cells into a 125 mL shaker flask.
4. For increased rate of successful cell revival, an initial viable cell density of at least 0.3 x 10^6 cells/mL in Step 3 is recommended. Determine viable cell density and cell viability using standard cell counting protocols.
5. Gently swirl the shake flask containing newly seeded cells and maintain cells in a 37 °C incubators, with relative humidity ≥ 80%, 5%

CO_2, and shaking at 120 ± 5 RPM on an orbital (19 – 25 mm) platform.

6. Subculture cells after 96 ± 2 hours in culture or when the viable cell density reaches 3.0 – 4.0 x 10^6 cells/mL, whichever comes first.
7. Subculture cells every 3 – 4 days by diluting with fresh medium to a density between 0.5 – 0.7 x 10^6 cells/mL.
8. Maintain cells in TheraPEAK 293-GT® Medium for at least 3 passages before AAV production.

General guidelines for AAV production with TheraPEAK 293-GT® Media System

Cell culture

It is recommended that cells used for AAV production have been cultured in TheraPEAK 293-GT® Medium for at least 3 passages. Cells should have a low overall passage number, be free from contamination, and show at least 95% viability. A passage number between 3 and 20 is recommended at the time of transfection. As a starting point, AAV production at the scale of 30 mL working volume in 125 mL shake flasks is recommended. Viable cell density at time of transfection may be controlled between 2.5 – 3.5 x 10^6 cells/mL or follow the recommendations provided for respective transfection reagents. Further optimization of cell density for individual cell lines may be beneficial and appropriate scale-up/scale-down is recommended after successfully establishing the process at 30 mL scale.

Plasmid DNA

TheraPEAK 293-GT® Media System is optimized for use in triple transfection workflows using transfer (containing the gene of interest), Rep/Cap, and Helper plasmids. Plasmid DNA used for transfection should have verified sequence accuracy, optical density at 260/280 nm between 1.8 – 2.0, endotoxin levels ≤0.01 EU/µg, and be 90% ± 10% supercoiled. The use of lower quality plasmid DNA is not recommended and will lead to less than desirable results. Plasmid preparations are assumed to be formulated in 1X TE buffer or double distilled (dd) H_2O.

Transfection

Three days prior to transfection, seed the cells in TheraPEAK 293-GT® Medium at 0.5 – 0.8 x 10^6 cells/mL. On the day of transfection, dilute cells with fresh TheraPEAK 293-GT® Medium to target cell density (recommended value is 2.5 – 3.5 x 10^6 cells/mL). Within 1 hour prior to next step, add TheraPEAK 293-GT® AAV Supplement to the diluted cell culture at a ratio of 1 mL per 30 mL cell culture.

For preparation of transfection complex, follow manufacturers' recommendations provided for respective transfection reagents.

Table 1 summarizes the general guideline parameter values for transfection with TheraPEAK 293-GT® Media System in 125 mL shake flasks. For best outcomes, optimize parameters as needed for individual cell lines, transfection reagents and plasmids.

Table 1. General guidelines of initial transfection parameters

Parameter	Standard value
Passages prior to transfection	3 to 20
Scale	125 mL Shake Flask
Culture volume [mL]	30
TheraPEAK 293-GT® Supplement [mL]	1
Shaking rate [rpm]	120 ± 5
pCO_2 [%]	5
Temperature [°C]	37
Inoculation cell concentration	2.5–3.5 x 10^6 cells/mL
Culture duration prior to transfection [hr]	72 ± 2
Production duration [hr]	72 ± 2

Harvest procedures

General cell lysis and AAV harvest procedures may apply. For further advice and recommendations, please contact Lonza Scientific Support Team.

Product list

Reagent	Catalog no.	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. All trademarks belong to 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. The user bears the sole responsibility for determining the existence of any third-party rights and 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: **http://www.lonza.com/legal, https://www.lonza.com/about-us/strategy/intellectual-property and http://www.lonza.com/privacy.**

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