Viral Production Cells 2.0 and Viral Production Medium

Catalog Numbers A49784, A51218, A4817901, A4817902, A4817903

Pub. No. MAN0019620 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Gibco™ Viral Production Cells 2.0 (VPCs 2.0) are a clonal derivative of the HEK293F cell line and have been adapted to suspension, high-density culture in Gibco™ Viral Production Medium. These cells can be thawed directly into Gibco™ Viral Production Medium.

Gibco™ Viral Production Medium is a chemically defined, serum-free, protein-free, animal origin-free medium developed for growth and transfection of VPCs 2.0. Prior to use, the medium requires supplementation with 4 mM GlutaMAX™ Supplement.

Contents and storage

Product	Cat. No.	Amount	Storage
Gibco™ Viral Production Cells 2.0	A49784	1 mL (1×10^7 cells/mL)	Liquid nitrogen
	A51218	6 × 1 mL (1×10^7 cells/mL)	
Gibco™ Viral Production Medium	A4817901	1 L bottle	2°C to 8°C, protect from light
	A4817902	6 × 1 L bottle	
	A4817903	10 L bag	

Culture conditions

Media: Viral Production Medium

Culture type: Suspension

Incubator atmospere: 37°C, ≥80% relative humidity, and 8% CO_2

Shaker speed: Set the shake speed to 125±5 rpm for shakers with a 19-mm shaking diameter, 120±5 rpm for shakers with a 25-mm shaking diameter and 95±5 rpm for shakers with a 50-mm shaking diameter.

Guidelines for handling cells

- **IMPORTANT!** Store the frozen cells in liquid nitrogen until ready to use. Do not store the cells at –80°C
- Avoid subjecting cells to short-term, extreme temperature changes.
- Store cells in liquid nitrogen following receipt on dry ice. Allow the cells to remain in liquid nitrogen for 3 to 4 days before thawing.
- For all cell manipulations, mix cells by gentle swirling and avoid vigorous shaking/pipetting.
- For routine cell culture maintenance, subculture cells every 3 to 4 days when they reach 4 to 6 × 10^6 cells/mL. Do not subculture cells that have not reached early log phase growth of ≥4 × 10^6 cells/mL.

Prepare medium

Supplement Viral Production Medium with 4 mM GlutaMAX™ Supplement for thaw, culture, cryopreservation, and viral production. GlutaMAX™ Supplement (Cat. No. 35050061) is formulated at 200 mM.

Thaw Viral Production Cells 2.0

1. Add 30 mL of pre-warmed Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement to a 125-mL Erlenmeyer shaker flask.
2. Remove a vial of Viral Production Cells 2.0 (VPCs 2.0) from liquid nitrogen and swirl gently in a 37°C water bath for 1 to 2 minutes to thaw the cells rapidly until only a small amount of ice remains.

   Note: Do not submerge the vial in the water.
3. Just before the cells are completely thawed, decontaminate the vial with 70% ethanol before opening it in a laminar flow hood.

4. Using a 2-mL or 5-mL pipette, transfer the entire contents of the cryovial into the shaker flask prepared in step 1 on page 1.

   Note: With a 2-mL pipette, gently mix the contents of the cryovial up and down once and then transfer 1.0 mL of the cells to the shake flask prepared in step 1. From the cell volume remaining in the cryovial, transfer 50 µL of cells into 450 µL of Ca^{2+}/Mg^{2+} free PBS. Use the diluted cells to determine viability. In certain instances, components in cell culture media can interact with trypan blue leading to aggregation that can be misinterpreted as dead cells using typical cell counting instruments and algorithms. It is recommended to utilize PBS as a diluent for counting cells and determining cell viability at the time of thaw to minimize this risk.

5. Incubate the cells in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker platform.

   Note: Set the shake speed to 125±5 rpm for shakers with a 19-mm shaking diameter, 120±5 rpm for shakers with a 25-mm shaking diameter and 95±5 rpm for shakers with a 50-mm shaking diameter.

6. After 3 to 4 days post-thaw, determine the viable cell density and percent viability. Cell viability should be ≥90% with a viable cell density >1 × 10^6 viable cells/mL.

   Note: If viability is <90% on days 3 to 4 post-thaw, cells may be cultured for up to an additional 3 days in order to reach the desired viability. Cells should not be subcultured until viable cell density reaches 1 to 3 × 10^6 viable cells/mL.

7. For subsequent routine cell culture maintenance, subculture cells every 3 to 4 days when the viable cell density reaches 4 to 6 × 10^6 viable cells/mL according to Table 1 and Table 2.

   Note: Do not subculture cells before reaching early log phase growth of ≥4 × 10^6 cells/mL. Similarly, do not let cells overgrow above ≥6.5 × 10^6 cells/mL.

Subculture Viral Production Cells 2.0

VPCs 2.0 are capable of achieving high cell densities; therefore, it is important that cells attain a minimum density of 4 to 6 × 10^6 viable cells/mL at the time of subculturing.

1. At the time of subculture, calculate viable cell density.

   Note: If using a Vi-CELL™ cell counting instrument, for the recommended settings for this cell line see Table 1 and Table 2.

2. Use the viable cell density to calculate the volume of cell suspension required to seed a new shaker flask according to the recommended seeding densities in Table 3 and the recommended culture volumes in Table 4.

Table 1 Recommended Vi-CELL™ XR cell counting settings

Parameter	Value	Parameter	Value
Minimum diameter	10	Cell brightness	85
Maximum diameter	30	Cell sharpness	100
Number of images	50	Viable cell spot brightness	65
Aspirate cycles	3	Viable cell spot area	5
Trypan blue mixing cycles	3	Minimum circularity	0
Decluster degree	Medium	—	—

Table 2 Recommended Vi-CELL™ BLU cell counting settings

Parameter	Value	Parameter	Value
Minimum diameter	10	Cell sharpness	7.0
Maximum diameter	30	Viable cell spot brightness	40
Number of images	100	Viable cell spot area	5
Aspirate cycles	6	Maximum cirularity	0.10
Trypan blue mixing cycles	6	—	—
Decluster degree	High	—	—

Table 3 Recommended seeding densities for routine cell culture maintenance

Sub-culture timing	Recommended seeding density
For cells ready 3 days post-subculture	0.6 × 10^6 viable cells/mL
For cells ready 4 days post-subculture	0.3 × 10^6 viable cells/mL

Note: Modify the initial seeding density to attain the target cell density of 4 to 6 × 10^6 viable cells/mL at the time of subculturing.

Table 4 Recommended volumes for routine cell culture maintenance

Flask size	Culture volume[1]	Shake speed
125 mL	30 mL	125±5 rpm (19-mm orbital diameter) 120±5 rpm (25-mm orbital diameter) 95±5 rpm (50-mm orbital diameter)
250 mL	60 mL	
500 mL	120 mL	
1 L	240 mL	
2 L	480 mL	
2.8 L	700–1000 mL	90±5 rpm (19-mm orbital diameter) 85±5 rpm (25-mm orbital diameter) 80±5 rpm (50-mm orbital diameter)

[1] If using volumes outside of the recommended range, it is critical to ensure that all cell growth (i.e., doubling times), health (i.e., cell diameter, viability), and production levels remain consistent with control conditions. Cell performance is decreased if cell health is compromised.

3. Transfer the appropriate number of cells to fresh, pre-warmed Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement in a shaker flask.

4. Incubate the flasks in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker platform until the cultures reach a density of 4 to 6 × 10^6 viable cells/mL.

   Note: Cells that are subcultured at densities outside of this early log-phase growth window may show longer doubling times and lower titers over time. Modify the initial seeding density to attain the target cell density of 4 to 6 × 10^6 viable cells/mL at the time of subculturing.

5. Repeat step 1 to step 3 to maintain or expand cells for transfection.

Cryopreserve Viral Production Cells 2.0

VPCs 2.0 can be frozen directly in Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement with 10% DMSO. Alternatively, conditioned cryopreservation medium consisting of 45% fresh Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement, 45% conditioned medium and 10% DMSO can be used.

1. Prepare the freezing medium with 90% Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement + 10% DMSO and place on ice until cells are ready to use.

2. Determine the viable cell density and calculate the volume of cell culture needed for cryopreservation.

3. Centrifuge the calculated amount of cell volume at 300 × *g* for 5 minutes. Discard the supernatant without disturbing cell pellet.

4. Add a smaller volume (10% of final banking volume) of cold freezing medium to the pellet, then gently resuspend the cell pellet by pipetting using a wide-bore pipette. Once resuspended, further dilute the cells to a final density of 1 × 10^7 viable cells/mL in freezing medium.

5. Freeze 1 mL of cells in an automated or manual controlled-rate freezing apparatus following standard procedures. For manual processes, freeze the cells at -80°C for overnight prior to transfer the frozen cells to liquid nitrogen. For ideal cryopreservation, the rate of temperature decrease should be 1°C per minute.

6. Transfer frozen the vials to liquid nitrogen for long-term storage.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
GlutaMAX™ Supplement	35050061
AAV-MAX Transfection Kit	A50515 A50516
Viral-Plex™ Complexation Buffer	A4983901
AAV-MAX Helper-Free AAV Production System Kit	A51217
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile	4115-0125
MaxQ™ HP Tabletop Orbital Shaker	SHKE416HP
Countess™ Automated Cell Counter	AMQAX2000
Trypan Blue Stain	T10282

Limited product warranty

Manufacturer:
Life Technologies Corporation |
5781 Van Allen Way |
Carlsbad, California 92008 USA

Product:
Viral Production Cells 2.0

Manufacturer:
Life Technologies Corporation |
3175 Staley Road |
Grand Island, New York 14072 USA

Product:
Viral Production Medium

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.