CTS™ Viral Production Cells 2.0

Catalog Number A48400

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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™ CTS™ Viral Production Cells 2.0 are a clonal derivative of the HEK293F cell line and have been adapted to suspension, high-density culture in CTS™ Viral Production Medium. These cells can be thawed directly into CTS™ Viral Production Medium. CTS™ Viral Production Medium is formulated with GlutaMAX™ Supplement and does not require further supplementation.

Cell line characteristics:

- Transformed via culture with sheared human adenovirus 5 DNA
- Does not contain the SV40 large T antigen
- Cell doubling time of <24 hours
- Achieves maximum cell densities of >1.2 × 10^7 cells/mL in shaker flask cultures
- Maximal AAV production can be achieved between cell passages 3 to 25

Contents and storage

Contents	Cat. No.	Amount	Storage
CTS™ Viral Production Cells 2.0	A48400	1 vial (1.5 mL)	Liquid nitrogen

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
125-mL Erlenmeyer flasks (e.g., Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile)	4115-0125
Orbital shaker: (e.g. MaxQ™ HP Tabletop Orbital Shaker)	SHKE416HP
Temperature and CO_2 controlled incubator (e.g. Large-Capacity Reach- In CO_2 Incubator)	3950, 3951, ETO
Reagents and equipment to determine cell viability (e.g., hemocytometer with trypan blue or cell counter)	MLS

Guidelines for handling cells

IMPORTANT! Store the frozen cells in liquid nitrogen immediately upon receipt 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.
- 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 the viable cell density reaches 4–6 × 10^6 cells/mL. Do not subculture cells that have not reached early log phase growth of ≤4 × 10^6 cells/mL.

Thaw CTS™ Viral Production Cells 2.0

1. Add 30 mL of pre-warmed CTS™ Viral Production Medium to a 125-mL Erlenmeyer shaker flask.

2. Remove a vial of CTS™ Viral Production Cells 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. Gently invert the cell vial to mix the contents. Uncap the cell vial and transfer 50 µL of cells into 450 µL of Ca^{2+}/Mg^{2+} free PBS for viability and viable cell density determination by trypan blue dye exclusion assay.

   Note: Trypan blue may interact with components in cell culture media leading to aggregation that can be misinterpreted as dead cells using typical cell counting instruments and algorithms. Dilution with PBS is not required during routine cell culture maintenance.

5. Using a 1-, 2-, or 5-mL pipette, gently transfer the remaining cell volume drop wise to the shake flask containing the pre-warmed culture medium prepared in step 1.

6. Incubate the cells in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker platform. The use of non-humidified incubators is not recommended due to the significant loss of volume in the culture flasks by evaporation.

   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.

7. Three to four 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 the 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 × 10^6 viable cells/mL.

8. 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 3.

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

Subculture CTS™ Viral Production Cells 2.0

CTS™ Viral Production Cells 2.0 are capable of achieving high cell densities. Therefore, it is important that cells attain a minimum density of 4– 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, refer to the recommended settings for this cell line listed in 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	Vialbe cell spot area	5
Trypan blue mixing cycle	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 brightness	5
Aspirate cycles	6	Minimum circularity	0.10
Trypan blue mixing cycle	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	0.5–0.7 × 10^6 viable cells/mL (target 0.6 × 10^6 viable cells/mL)
For cells ready 4 days post-subculture	0.3–0.5 × 10^6 viable cells/mL (target 0.4 × 10^6 viable cells/mL)

Note: Modify the initial seeding density to attain the target cell density of 4–6 × 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)
250 mL	60 mL	120 ±5 rpm (25-mm orbital diameter)
500 mL	120 mL	
1 L	240 mL	95 ±5 rpm (50-mm orbital diameter)
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 CTS™ Viral Production Medium 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 CTS™ Viral Production Cells 2.0

CTS™ Viral Production Cells 2.0 can be frozen directly in CTS™ Viral Production Medium with 7.5% DMSO. Alternatively, conditioned cryopreservation medium consisting of 42.5% fresh CTS™ Viral Production Medium, 50.0%, conditioned medium and 7.5% DMSO can be used.

Note: It is critical that chemical compatibility be maintained throughout the freezing process to eliminate the potential for plastics leachables/extractables to negatively impact cell health. For all steps where DMSO is present (with exception only to the pipet tips used for aliquoting the final cell solution into cryo-vials), glass serological pipettes are suggested to be used, as polystyrene is generally not compatible with concentrated DMSO. Similarly, DMSO containing freeze medium must be prepared in polypropylene or other known DMSO compatible bottles and the final cell suspension in freeze medium must be prepared in polypropylene or other known DMSO compatible bottles prior to aliquoting into polypropylene freeze tubes. Additionally, all pipets and bottles may be rinsed with sterile water, PBS or culture medium before use as desired. Refer to https://tools.thermofisher.com/content/sfs/brochures/D20480.pdf for guidance on chemical compatibility.

1. Determine the density and volume of cells required for banking cells at a final density of 1 × 10^7 viable cells/mL in 1.1 mL total volume. Expand the cells, maintaining a viable cell density of 4–6 × 10^6 viable cells/mL, until the desired volume of cells to be banked is ready. Do not use shake flasks larger than 2 L to culture the cells, as these flasks differ in shape and require altered shaking speeds and be sure to adhere to the shaking speed, orbital diameter and maximum flask volume recommendations.

2. While expanding the cells for banking, prepare an additional flask which will be used to generate conditioned medium. This flask should be prepared and expanded in the same manner as the flasks used for cell banking.

   Note: Based on the total volume of the bank, prepare at least ½ volume conditioned medium (i.e., If total volume for cell banking is 100 mL, at least 50 mL of conditioned medium will be required).

3. Prepare labels and label the appropriate number of vials. If vials are labeled on a day other than that of the harvest, store vials in a biosafety cabinet.

   Note: To reduce the risk of damaging the cells during freezing procedures, cells pellets will be resuspended in cold, 100% conditioned medium followed by 1:1 addition of cold freeze medium with 15% DMSO to reach a final concentration of 7.5% DMSO, 50% conditioned medium, and 42.5% fresh medium. It is critical to resuspend the cell pellets in cold medium free of DMSO.

   Note: If use of 100% fresh culture medium for banking cells is desired, cells pellets will be resuspended in cold, 100% fresh medium followed by a 1:1 addition of cold, 15% DMSO in fresh cell culture medium to reach a final concentration of 7.5% DMSO and 92.5% fresh medium.

Prepare conditioned medium

All conditioned medium is to be pre-chilled before use in cell banking.

1. Remove conditioned medium flask from incubator and transfer the entire volume of cell suspension into a sterile polypropylene centrifuge tube or bottle.

2. Centrifuge the cells at 300 × *g* for 10 minutes at 2–8°C.

3. Carefully decant the supernatant into a sterile polypropylene bottle without disturbing the cell pellet; the decanted supernatant will be used as the conditioned medium.

4. Store the conditioned medium in a 2–8°C refrigerator or on ice for a minimum of 2 hours.

5. Discard the cell pellets.

Prepare freeze medium (2X)

1. In a sterile polypropylene bottle, prepare the required amount of fresh culture medium supplemented with 15% DMSO. This represents a 2X freeze medium. It is recommended to use glass serological pipettes for transferring the concentrated DMSO to the culture medium. Keep 2X DMSO freeze medium cold at 2–8°C or on ice until use.

2. Remove calculated volume of cells from incubation and transfer into sterile polypropylene centrifuge bottle/tube. Centrifuge the cells at 200 × *g* for 10 minutes at 2–8°C. Carefully decant the supernatant without disturbing the cell pellets. After removing the supernatant, gently flick the bottom of the centrifuge tube to loosen the cell pellet.

3. Resuspend the cell pellet by gently pipetting with ~10% volume of conditioned medium using a wide bore pipet (i.e., If total bank volume is 200 mL, use 20 mL to resuspend cell pellet).

4. Add additional conditioned medium to the centrifuge bottle to obtain a 2X cell stock. For example, if banking at 1×10^7 cells/mL prepare a 2X cell stock at 2×10^7 cells/mL. Gently swirl the bottle to ensure a homogenous mixture and keep cold on ice or cold blocks.

   Note: It is critical that the next steps are performed as quickly as possible to limit the DMSO exposure time.

5. Using a glass serological pipette, add the calculated volume of cold, 2X freeze medium to the bottle containing 2X cell stock in conditioned medium from “Prepare conditioned medium” on page 3.

6. Gently swirl the bottle to ensure a homogenous mixture and keep cold on ice or cold blocks.

   At this point the volume should be equal to the total bank volume at a final concentration of 7.5% DMSO, 50% conditioned medium, and 42.5% fresh medium with a cell density of 1×10^7 cells/mL.

7. Immediately dispense a 1.1 mL aliquot of the final cell suspension from step 6 into labeled cryo-vials using a repeater pipette or sterile serological pipettes.

8. Gently swirl the cell suspension to mix before each refill of the multi-dispenser pipette or serological pipette. Make sure to keep the cell suspension cold during the entire aliquoting process.

   Note: The DMSO in the 2X freeze medium is harmful to the cells so it is important to limit the amount of DMSO exposure prior to freezing. We recommend keeping the DMSO exposure time ≤ 60 minutes as possible and keeping all reagents cold during this time.

9. Transfer the cryo-vials to isopropanol containing cryo-freezing chambers and store at -80°C for 24–48 hours. Do not store cells at -80°C for more than 48 hours. After 24–48 hours transfer cells to final storage in vapor phase liquid nitrogen.

   This freezing regimen approximates a 1°C per minute cooling in the isopropanol containers to -80°C followed by a 2°C per minute cooling in vapor phase liquid nitrogen to final storage temperature. These cooling conditions may be utilized as a basis for controlled rate freezing protocol design.

Transfect CTS™ Viral Production Cells 2.0

For detailed instructions on AAV production, see *CTS™ AAV-MAX Helper-Free AAV Production System User Guide* (Pub. No. MAN0026650) at **thermofisher.com**.

Related products

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

Catalog numbers that appear as links open the web pages for those products.

Item	Source
CTS™ Viral Production Medium	A5144001, A5144002, A5144003, A5416001, A5416002
Viral Production Medium, AGT	A5147101, A5147102, A5147103, A5147104
CTS™ AAV-MAX Transfection Kit	A5427701, A5427702, A5427703
CTS™ Viral-Plex™ Complexation Buffer	A5145401, A5145402, A5145403
AAV-MAX Lysis Buffer	A50520

Limited product warranty

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

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Revision	Date	Description
1.0	11 October 2022	New document for CTS™ Viral Production Cells 2.0.