

CTS™ Viral Production Cells

For establishment of CTS™ Viral Production Cells

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Note: For safety and biohazard guidelines, see the “Safety” appendix in the *CTS™ LV-MAX™ Lentiviral Production System User Guide* (Pub. No. MAN0018450). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The CTS™ Viral Production Cells are a derivative of the original HEK293F cell line that have been adapted to suspension culture in LV-MAX™ Production Medium. These cells can be thawed directly into CTS™ LV-MAX™ Production Medium.

Cell line characteristics:

- Transformed via culture with sheared human adenovirus 5 DNA
- Expresses E1A adenoviral gene
- Lacks the SV40 large T antigen
- Cell doubling time of ~26 hours
- Achieves maximum cell densities of $\sim 1 \times 10^7$ cells/mL in shaker flask cultures
- High lentiviral production capabilities between cell passages 5–20

Procedural guidelines

- All solutions and equipment that come in contact with cells must be sterile.
- Thaw and transfer cells directly into a 125-mL shaker flask with 30 mL of pre-warmed 37°C CTS™ LV-MAX™ Production Medium. Centrifugation and medium change are not required when seeding the cells.
- Do not centrifuge the cells as this can lower cell viability.
- Culture cells for 3–4 days post-thaw and then determine viable cell density and percent viability. Cell viability should be $\geq 90\%$ 3–4 days post-thaw, with viable cell density typically $> 1 \times 10^6$ viable cells/mL; if viability is not $> 90\%$, incubate cells for up to an additional 3 days to reach optimal density. Perform the first subculture when the viable cell density reaches $1\text{--}3 \times 10^6$ viable cells/mL (typically 4–7 days post-thaw).
- Subculture CTS™ Viral Production Cells when they reach a density of $3.5\text{--}5.5 \times 10^6$ viable cells/mL (i.e., early log-phase growth), typically every 3–4 days.

Note: Do not let cells grow above 5.5×10^6 viable cells/mL during routine culture.

Note: Cells subcultured at densities outside of the 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 $3.5\text{--}5.5 \times 10^6$ viable cells/mL at the time of subculturing.

- Allow freshly thawed cells to recover in culture for 5 passages post-thaw before using cells for virus production.
- Inspect cells to ensure that round cell morphology is maintained over passages for most of the cell population with minimal cell clumping. Proper cell phenotype is critical for optimal transfection and virus production.
- When thawing or subculturing cells, transfer cells into pre-warmed medium.
- It is recommended to discard cells and start a new culture after passage number 20.

- The following table shows the recommended cell density for routine cell culture:

Table 1 Recommended seeding densities for routine cell culture maintenance

Sub-culture timing	Recommended seeding density
For cells ready 3 days post-subculture	$0.4\text{--}0.6 \times 10^6$ viable cells/mL
For cells ready 4 days post-subculture	$0.2\text{--}0.4 \times 10^6$ viable cells/mL

- The following table shows the recommended shaker speed and culture volume for cultivation:

Table 2 Recommended volumes for routine cell culture maintenance in vented, non-baffled flasks

Flask size	Culture volume [1]	Parameter
125 mL	30–35 mL	125±5 rpm (19 mm shaking diameter) 120±5 rpm (25 mm shaking diameter) 95±5 rpm (50 mm shaking diameter)
250 mL	60–70 mL	
500 mL	120–140 mL	
1 L	240–280 mL	
2 L	480–560 mL	90±5 rpm 85±5 rpm 80±5 rpm
2.8–3 L	720–840 mL	

[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 expression levels remain consistent with control conditions. Cell performance is decreased if cell health is compromised.

Thaw and establish CTS™ Viral Production Cells

Week 1: P0–P1

1. Passage 0 (P0): Remove one vial of cells from liquid nitrogen, then swirl 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.
2. Just before the cells are completely thawed, decontaminate the vial by wiping it with 70% ethanol before opening it in a laminar hood.
Note: Centrifugation and medium change are not required for these cells.
3. Use a 2-mL or 5-mL pipette to transfer the entire contents of the cryovial into a 125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask containing 30 mL of CTS™ LV-MAX™ Production Medium pre-warmed to 37°C.
4. Incubate the cells in a 37°C incubator with ≥80% relative humidity and 8% CO₂ on an orbital shaker platform.
Note: Set the shaker 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.
5. Passage 1 (P1): Seed cells at a density of 0.5 × 10⁶ cells/mL in a total culture volume of 30 mL in a new 125-mL shaker flask.

Week 2: P2–P3

6. Passage 2 (P2): Seed cells at a density of 0.3 × 10⁶ cells/mL in a total culture volume of 30 mL in a new 125-mL shaker flask.
7. Passage 3 (P3): Seed cells at a density of 0.5 × 10⁶ cells/mL in a total culture volume of 30 mL in a new 125-mL shaker flask.

Week 3: P4–P5

8. Passage 4 (P4): Seed cells at a density of 0.3 × 10⁶ cells/mL in a total culture volume of 60–70 mL in a new 250-mL shaker flask.
9. Passage 5 (P5): Seed cells at a density of 0.5 × 10⁶ cells/mL in 240–280 mL of total culture volume in a 1-L shaker flask to ensure appropriate cell numbers for lentivirus production.

Week 4: Cells are established and ready for lentivirus production

10. Continue to subculture cells when required until P20. Proceed with lentiviral production.

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