

CTS™ LV-MAX™ Lentiviral Production System

USER GUIDE

For xeno-free lentiviral vector production in suspension cells

for use with:

CTS™ Viral Production Cells

CTS™ LV-MAX™ Production Medium

CTS™ LV-MAX™ Transfection Kit

CTS™ Opti-MEM™ I

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Revision B.0

For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products. CAUTION: Not intended for direct administration into humans or animals.



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B.0	5 January 2021	Update of reagent volumes in protocol.
A.0	28 March 2019	New document.

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Product information

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

The CTS™ LV-MAX™ Lentiviral Production System is a lentiviral vector production system based on transient transfection of high-density, suspension HEK293F cells adapted to a chemically defined, serum-free and protein free medium. The system is available in both Research Use and Cell Therapy Systems (CTS) options to enable a seamless transition from discovery to production.

The CTS™ LV-MAX™ Lentiviral Production System provides cells, production medium, supplement, transfection reagent, and enhancer to produce high titer lentiviral vectors.

Required components

The workflows described in this document are for use with the components in the following table.

Component	Cat. No.	Amount	Storage
CTS™ Viral Production Cells (1 X 10 ⁷ cells/mL)	A3152801	1 × 1 mL	Liquid nitrogen ^[1]
CTS™ LV-MAX™ Production Medium	<ul style="list-style-type: none">• A4124001• A4124004• A4124002• A4124003	<ul style="list-style-type: none">• 1 L• 6 × 1 L• 10 L• 20 L	<ul style="list-style-type: none">• 2°C to 8°C• Protected from light
One of the following CTS™ LV-MAX™ Transfection Kit:			
CTS™ LV-MAX™ Transfection Kit: <ul style="list-style-type: none">• CTS™ LV-MAX™ Supplement• CTS™ LV-MAX™ Transfection Reagent• CTS™ LV-MAX™ Enhancer	A4132601	1 L Production Kit <ul style="list-style-type: none">• 50 mL• 6 mL• 40 mL	
CTS™ LV-MAX™ Transfection Kit: <ul style="list-style-type: none">• CTS™ LV-MAX™ Supplement• CTS™ LV-MAX™ Transfection Reagent• CTS™ LV-MAX™ Enhancer	A4132602	10 L Production Kit <ul style="list-style-type: none">• 500 mL• 60 mL• 400 mL	

(continued)

Component	Cat. No.	Amount	Storage
CTS™ Opti-MEM™ I	• A4124801	• 100 mL	<ul style="list-style-type: none"> • 2°C to 8°C • Protected from light
	• A4124802	• 500 mL	

[1] Store the frozen cells in liquid nitrogen until ready to use. Do not store the cells at -80°C.

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.

Table 1 Materials required for lentiviral vector production (all methods)

Item	Source
CO ₂ resistant orbital shaker	88881101
125-mL shaker flasks for Viral Production Cells	MLS
Adjustable micropipettors	MLS
Laboratory mixer (Vortex mixer or equivalent)	MLS
Equipment and reagents to determine cell density and viability	MLS
LV-MAX™ Lentiviral Packaging Mix	A43237
For cryopreservation:	
DMSO	MLS
Cryovials	MLS

Table 2 Additional materials required for lentiviral vector production in a 96-deep well block

Item	Source
3 mm orbital shaker	MLS
2-mL sterile 96-deep well block	MLS
2-mL sterile 96-deep well block (V bottom)	MLS
96-well round bottom plate	MLS
Multi-channel micropipettors	MLS
PureLink™ Air Porous Tape	12262010

Table 3 Additional materials required for lentiviral vector production in a 50-mL conical tube

Item	Source
Nunc 50-mL conical tube	339653
Corning™ Mini Bioreactor Centrifuge Tube	07-202-150
50-mL conical tube holder	MLS
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile	
125 mL	4115-0125
250 mL	4115-0250
1 L	4115-1000

Table 4 Additional materials required for lentiviral vector production in a shaker flask

Item	Source
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile	
125 mL	4115-0125
250 mL	4115-0250
1 L	4115-1000
2 L	4115-2000

System components

The following section provides descriptions of the components in the CTS™ LV-MAX™ Lentiviral Production System.

Viral production cells

CTS™ Viral Production Cells are derived from the HEK293F cell line, and are adapted to suspension culture in CTS™ 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

Growth medium

CTS™ LV-MAX™ Production Medium is a complete, chemically defined, animal origin-free, serum-free, protein-free formulation, developed for growth and transfection of CTS™ Viral Production Cells. This medium is ready-to-use and does not require the addition of supplements.

Transfection supplement

CTS™ LV-MAX™ Supplement is a chemically defined, animal origin-free, serum-free, protein-free formulation designed to control cell growth during transfection and increase lentiviral vector production without compromising cell viability.

Transfection reagent

CTS™ LV-MAX™ Transfection Reagent is uniquely designed for high efficiency co-transfection of multiple plasmids into CTS™ Viral Production Cells, with low toxicity.

Production enhancer

CTS™ LV-MAX™ Enhancer is a chemically defined, animal origin-free, serum-free, protein-free formulation that is designed to boost lentiviral vector production in CTS™ Viral Production Cells.

Opti-MEM

CTS™ Opti-MEM™ I is a xeno-free formulation for use with transfection reagent to facilitate complexation in viral vector production. It is an improved Minimal Essential Medium (MEM) containing insulin, transferrin, hypoxanthine, thymidine, and trace elements.



Thaw and establish CTS™ Viral Production Cells

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.
- After storing cells in liquid nitrogen following receipt on dry ice, allow the cells to remain in liquid nitrogen for 3–4 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 3–4 days when they reach $3.5\text{--}5.5 \times 10^6$ cells/mL (see “Subculture CTS™ Viral Production Cells” on page 10). Do not subculture cells that have not reached early log phase growth of $\geq 3.5 \times 10^6$ cells/mL.

Required materials not supplied

- CTS™ Viral Production Cells
- 125-mL Erlenmeyer Flask (e.g., Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile for culturing Viral Production Cells, Cat. No. 4115-0125)
- Orbital shaker (e.g., MaxQ™ HP Tabletop Orbital Shaker, Cat. No. SHKE416HP)
- Temperature and CO₂ controlled incubator (e.g., Large-Capacity Reach-In CO₂ Incubator, Cat. No. 3950)
- Reagents and equipment to determine cell viability (e.g., hemocytometer with trypan blue or cell counter)

Thaw CTS™ Viral Production Cells

1. Remove the vial of cells from liquid nitrogen and 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 flow hood.
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 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.

5. Culture cells for 3–4 days post-thaw and then determine viable cell density and percent viability. Cell viability should be ≥90% 3–4 days post-thaw, with viable cell density typically >1 × 10⁶ viable cells/mL; if viability is not >90%, incubate cells for up to an additional 3 days to reach optimal density. Subculture cells when the viable cell density reaches 1–3 × 10⁶ viable cells/mL.

Subculture CTS™ Viral Production Cells

CTS™ Viral Production Cells are capable of achieving high cell densities; therefore, it is important that cells attain a minimum density of 3.5–5.5 × 10⁶ viable cells/mL at the time of subculturing.

Required materials

- CTS™ Viral Production Cells cultured in CTS™ LV-MAX™ Production Medium
- CTS™ LV-MAX™ Production Medium, pre-warmed to 37°C
- CTS™ Opti-MEM™ I
- Disposable, sterile Erlenmeyer flasks
- Orbital shaker (e.g., MaxQ™ HP Tabletop Orbital Shaker, Cat. No. SHKE416HP)
- Temperature and CO₂ controlled incubator (e.g., Large-Capacity Reach-In CO₂ Incubator, Cat. No. 3950)
- Reagents and equipment to determine cell viability (e.g., hemocytometer with trypan blue or cell counter)

Passage CTS™ Viral Production Cells

1. Use the viable cell density to calculate the volume of cell suspension required to seed a new shake flask according to the recommended seeding densities in Table 5 and the recommended culture volumes in Table 7.

Table 5 Recommended seeding densities for routine cell culture maintenance

Sub-culture timing	Recommended seeding density
For cells ready 3 days post-subculture	0.5×10^6 viable cells/mL
For cells ready 4 days post-subculture	0.3×10^6 viable cells/mL

Table 6 Recommended Vi-CELL™ XR Cell Counting Settings

Parameter	Value	Parameter	Value
Minimum diameter	5	Cell brightness (%)	85
Maximum diameter	50	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 7 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	
2.8–3 L	720–840 mL	90±5 rpm 85±5 rpm 80±5 rpm

^[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.

2. Transfer the calculated volume of cells to fresh, pre-warmed CTS™ LV-MAX™ Production Medium in a shake flask.

3. Incubate flasks in a 37°C incubator with ≥80% relative humidity, and 8% CO₂ on an orbital shaker platform until cultures reach a density of 3.5–5.5 × 10⁶ viable cells/mL.

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 3.5–5.5 × 10⁶ viable cells/mL at the time of subculturing.

4. Repeat Steps 1–3 to maintain or expand cells for transfection.

Cryopreserve CTS™ Viral Production Cells

CTS™ Viral Production Cells can be frozen directly in CTS™ LV-MAX™ Production Medium with 10% DMSO. Alternatively, conditioned cryopreservation medium consisting of 45% fresh CTS™ LV-MAX™ Production Medium, 45% conditioned CTS™ LV-MAX™ Production Medium, and 10% DMSO can be used.

Cryopreserve cells

1. Centrifuge cells that have attained a viable cell density of 3.5–5.5 × 10⁶ viable cells/mL and cell viability at 300 × *g* for 5 minutes. Discard the supernatant without disturbing the cell pellet.
2. Add ice cold CTS™ LV-MAX™ Production Medium with 10% DMSO to the cell pellet, then resuspend the cell pellet by gentle pipetting.
3. Dilute the cells to a final density of 1 × 10⁷ viable cells/mL in 1 mL total volume of 90% fresh CTS™ LV-MAX™ Production Medium with 10% DMSO.
4. Freeze the cells in an automated or manual controlled-rate freezing apparatus following standard procedures.
For ideal cryopreservation, the freeze rate should decrease by 1°C per minute.
5. Transfer the frozen vials to liquid nitrogen for long-term storage.



Produce lentiviral vector

Procedural guidelines

- Allow freshly thawed cells to recover in culture for three or more passages post-thaw before transfection.
- During all cell manipulations, mix the cells by gentle swirling; avoid vigorous mixing/pipetting. Cell health is critical to maximal performance.
- Gently invert the Transfection Reagent 4–5 times before use to ensure thorough mixing.
- Complexation of plasmid DNA and Transfection Reagent takes place at room temperature.
- To ensure sterility, DNA can be filtered through a 0.22- μm filter before use.
- See Table 8 for transfection at various scales.

Equipment guidelines

- It is critical that shaking diameter, shaking speed, flask size/type, and volume of culture to be transfected matches the recommendations in this protocol for both routine subculture and viral production runs for optimal performance.
- Humidified incubators ($\geq 80\%$ relative humidity) are recommended to reduce evaporation during expression runs. When using multi-well plates, use high-humidity settings if available, as evaporation is of greater concern.
- Ensure equipment is calibrated for temperature. In some instances, the total heat from the incubator and the shaker can cause cell culture temperatures to exceed the recommended ranges and lead to decreased cell growth, clumping or cell death. In such instances, reduce the temperature setting of the incubator to compensate for heat generated by the shaker.
- Ensure that equipment is calibrated for CO_2 . Levels of CO_2 should not exceed 8%.

Required materials

- CTS™ Viral Production Cells cultured in CTS™ LV-MAX™ Production Medium
- CTS™ LV-MAX™ Production Medium, pre-warmed to 37°C
- CTS™ Opti-MEM™ I
- Disposable, sterile Erlenmeyer flasks
- Orbital shaker (e.g., MaxQ™ HP Tabletop Orbital Shaker, Cat. No. SHKE416HP)
- Temperature and CO₂ controlled incubator (e.g., Large-Capacity Reach-In CO₂ Incubator, Cat. No. 3950)
- Reagents and equipment to determine cell viability (e.g., hemocytometer with trypan blue or cell counter)

Optimized transfection conditions

Condition	Amount
Total CTS™ Opti-MEM™ I	2 × 5% of lentivirus production volume
Total DNA per mL of lentivirus production	2.5 µg/mL (LPM + LTV)
Ratio of lentiviral packaging mix (LPM) to lentiviral transfer vector (LTV)	3 to 2
CTS™ LV-MAX™ Transfection Reagent	6 µL/mL of lentivirus production volume
CTS™ LV-MAX™ Enhancer	4% of the lentivirus production volume
CTS™ Viral Production Cells	4 × 10 ⁶ cells/mL production volume
Total CTS™ LV-MAX™ Supplement	5% of lentivirus production volume

Transfect CTS™ Viral Production Cells

Use 2.5 µg total plasmid DNA per mL of culture volume to be transfected. A 3:2 ratio of lentiviral packaging plasmid (LPM) to lentiviral transfer plasmid (LTV), this translates to 1.5 µg of packaging plasmid to 1 µg of transfer plasmid.

Table 8 Recommended volumes for transfection at various scales

Vessel type	96-deep well plate	24-deep well plate	Mini bioreactor tube	125-mL flask	250-mL flask	1-L flask	2-L flask	3-L flask
No. of cells required	3.84×10^6	12×10^6	72×10^6	1.2×10^8	2.4×10^8	9.6×10^8	19.2×10^8	38.4×10^8
Starting culture volume	816 µL	2.55 mL	15.3 mL	25.5 mL	51 mL	204 mL	408 mL	816 mL
Shake speed ^[1]	900±50 rpm (3 mm orbital shaking diameter)	225±5 rpm 250±5 rpm 235±5 rpm	240±5 rpm 250±5 rpm 245±5 rpm	125±5 rpm (19 mm orbital shaking diameter) 120±5 rpm (25 mm orbital shaking diameter) 95±5 rpm (55 mm orbital shaking diameter)				90±5 rpm 90±5 rpm 55±5 rpm
Total amount plasmid DNA (LPM + LTV) ^[2]	2.5 mg total plasmid DNA (LPM + LTV) per mL of culture volume for transfection (starting culture volume)							
Volume of LPM DNA ^[3]	1.44 µL	4.5 µL	27 µL	45 µL	90 µL	360 µL	720 µL	1.44 mL
Volume of LTV DNA ^[3]	0.96 µL	3 µL	18 µL	30 µL	60 µL	240 µL	480 µL	960 µL
Volume of CTS™ Opti-MEM™ I to dilute plasmid DNA ^[4]	48 µL	150 µL	900 µL	1.5 mL	3 mL	12 mL	24 mL	48 mL
CTS™ LV-MAX™ Transfection Reagent	5.76 µL	18 µL	108 µL	180 µL	360 µL	1.44 mL	2.88 mL	5.76 mL
Volume of CTS™ Opti-MEM™ I to dilute transfection reagent ^[5]	48 µL	150 µL	900 µL	1.5 mL	3 mL	12 mL	24 mL	48 mL
CTS™ LV-MAX™ Supplement	48 µL	150 µL	900 µL	1.5 mL	3 mL	12 mL	24 mL	48 mL
CTS™ LV-MAX™ Enhancer	38.4 µL	120 µL	720 µL	1.2 mL	2.4 mL	9.6 mL	19.2 mL	38.4 mL
Final production volume ^[6]	960 µL	3.0 mL	18 mL	30 mL	60 mL	240 mL	480 mL	960 mL

^[1] Recommended shake speed ranges; optimal shake speed should be determined empirically based on the specific laboratory equipment used.

^[2] As an option, the amount of packaging plasmid and transfer plasmid can be halved, but the change should be tested to determine the effect on transfection efficiency.

^[3] Assuming a plasmid DNA stock concentration of 1 mg/mL and a final concentration of 2.5 µg plasmid DNA (LPM + LTV) per mL of culture to transfect.

^[4] Volume used to dilute plasmid DNA (LPM + LTV).

^[5] Volume used to dilute CTS™ LV-MAX™ Transfection Reagent.

^[6] Production volume minus CTS™ LV-MAX™ Enhancer

Transfect cells

See Table 8 for recommended volumes for transfection at various scales.

1. Subculture and expand cells until they reach a density of $3.5\text{--}5.5 \times 10^6$ viable cells/mL.

Split cells (Day –1)

2. One day before performing transfection, dilute the cells from Step 1 to a final density of 3.5×10^6 viable cells/mL and allow the cells to grow overnight.

Transfect cells (Day 0)

3. Determine viable cell density and percent viability. Cells should have reached a density of approximately $5.0\text{--}6.0 \times 10^6$ viable cells/mL. Viability should be $\geq 95\%$ to proceed with transfection.
4. Dilute the cells from Step 3 to a final density of 4.7×10^6 viable cells/mL with fresh CTS™ LV-MAX™ Production Medium followed by addition of CTS™ LV-MAX™ Supplement. Swirl the flasks gently to mix the cells.

Note: After the addition of all reagent's cells are transfected at a final density of 4.0×10^6 viable cells/mL.

Note: Discard the remaining cells; do not re-use high-density cells for routine subculturing.

5. Prepare CTS™ LV-MAX™ Transfection Reagent/plasmid DNA complexes.
 - a. Gently invert the bottle of CTS™ LV-MAX™ Transfection Reagent 4–5 times to mix.
 - b. Dilute plasmid DNA with Opti-MEM. Mix by swirling the tube and/or by inversion.
 - c. Dilute CTS™ LV-MAX™ Transfection Reagent with Opti-MEM. Mix by swirling the tube and/or by inversion or gentle pipetting 2–3 times.
 - d. Add the diluted plasmid DNA (from 5b) to the diluted CTS™ LV-MAX™ Transfection Reagent (from 5c). Mix by swirling the tube and/or by inversion or gentle pipetting 2–3 times.
6. Incubate CTS™ LV-MAX™ Transfection Reagent/plasmid DNA (from Step 5d) at room temperature for 10 minutes, and then slowly transfer 10% of the final production volume (e.g., 3 mL for a 30 mL final production volume) to the shaker flask, swirling the flask gently during addition.

Note: The CTS™ LV-MAX™ Transfection Reagent/plasmid DNA is stable for up to 1.5 hours.

7. Incubate the cells in a 37°C incubator with a humidified atmosphere of 8% CO₂ in air on an orbital shaker (for suggested shake speeds, see Table 7).
8. Five to six hours post transfection, add CTS™ LV-MAX™ Enhancer (e.g., 1.2 mL CTS™ LV-MAX™ Enhancer if transfecting 30 mL of cells).

Note: CTS™ LV-MAX™ Enhancer can be added up to a maximum of 14 hours post transfection.

Harvest the lentiviral vector

1. Harvest the lentiviral vector 48–55 hours post-transfection.
 - a. Collect the culture medium, then centrifuge the cells at $1,300 \times g$ in a swinging bucket centrifuge for 15 minutes.

Note: For smaller volumes, use a 1.5 mL microcentrifuge tube and centrifuge in a benchtop centrifuge at 13,000 rpm for 5 minutes.

- b. Harvest the lentiviral vector by transferring the supernatant to a fresh tube.
 - c. Further remove cell debris by filtration through a $0.45 \mu\text{m}$ low protein binding filter.
2. Immediately following harvest, store the lentiviral vector at -80°C .

Note: Prepare aliquots of the supernatant to avoid multiple freeze thaw cycles.

Titer the lentiviral vector

Thaw, then titer the lentiviral vector using your method of choice (see Chapter 5, “Titer lentiviral vector”).

Note: Thaw the virus on ice before use.

4

Produce recombinant lentiviral vector in a 3-L stirred tank bioreactor

Procedural guidelines

- For optimal performance, it is critical that the cell density, shaking diameter, shaking speed, flask size/type and volume of culture to be transfected match the recommendations in Table 5 and Table 7 for routine maintenance of cells.
- Ensure all equipment is calibrated prior to use. Out of specification temperature, gas volumes, and pH control can negatively affect the system and lead to reduced titers, decreased cell growth, clumping or cell death.

Guidelines for scaling up reactions

- When scaling up to vessels with a >2 L working volume, the power input per volume (P/V), tip speed, mixing time, and addition of the transfection complex must be taken into consideration. The P/V for the listed protocol is 4.5 W/m³ and the tip speed is 0.4 m/s. This provides a robust system at the 2 L working volume scale that minimizes shear from the impeller while allowing for optimal mixing. It is recommended to scale up using P/V when possible.
- Ensure proper attention is given to hold time and mixing of transfection reagents, DNA, and the transfection complex. Plan ahead to ensure each step is thoroughly mixed and timing of incubation/addition is not prolonged unnecessarily.
- A decrease in titer is not uncommon with increased scale. Minimizing hold and addition times, ensuring proper mixing of the transfection complex, and optimization of process parameters helps to minimize decreases in titer.

Required materials

- CTS™ LV-MAX™ Transfection Kit
- CTS™ Viral Production Cells cultured in CTS™ LV-MAX™ Production Medium
- Plasmid DNA preparation, sterile, free from phenol and sodium chloride, and containing mostly supercoiled DNA

Note: Isolate plasmid DNA using the PureLink™ HiPure Plasmid Kit(see “Related products” on page 33 for ordering information)

- CTS™ Opti-MEM™ I

- CTS™ LV-MAX™ Production Medium, pre-warmed to 37°C
- Disposable, sterile Erlenmeyer flasks
- Orbital shaker (e.g., MaxQ™ HP Tabletop Orbital Shaker, Cat. No. SHKE416HP)
- Temperature and CO₂ controlled incubator (e.g., Large-Capacity Reach-In CO₂ Incubator, Cat. No. 3950)
- Reagents and equipment to determine cell viability (e.g., hemocytometer with trypan blue or cell counter)
- 3-L HyPerforma™ Glass Stir Tank Bioreactor with HyPerforma™ G3Lab Controller or comparable
- Nalgene™ PETG bottles with transfer caps or other transfer bottles
- Sterile tube welder
- Reagents and equipment to determine gas concentrations, pH, and metabolites

Transfect CTS™ Viral Production Cells

Use 2.5 mg total plasmid DNA per liter of culture volume to be transfected. A 3:2 ratio of lentiviral packaging plasmid (LPM) to lentiviral transfer plasmid (LTV).

Table 9 Recommended volumes for 3 L stirred tank bioreactor

Reagent	Volume
Culture volume	1.6 L
Plasmid DNA	2.5 mg total plasmid DNA per liter of culture volume
Volume of plasmid DNA (LPM + LTV) ^[1]	4 mL
Volume CTS™ Opti-MEM™ I required to dilute plasmid DNA	80 mL
CTS™ LV-MAX™ Transfection Reagent	9.6 mL
Volume Opti-MEM required to dilute transfection reagent	80 mL
CTS™ LV-MAX™ Supplement	64 mL
CTS™ LV-MAX™ Enhancer	80 mL
Final volume	~1.9 L

^[1] Assuming a plasmid DNA stock concentration of 1 mg/mL.

Table 10 Recommended bioreactor settings

Reagent	Volume
Temperature	37°C ± 0.5
Working volume	2 L
Sparger	L-shaped drilled hole macrosparge
Impellers	1 × Rushton (bottom), 1 × three pitched blade (top)
Impeller diameter	55 mm
Impeller power number	1.4
Agitation	140 rpm, P/V 4.5 W/m ³ , tip speed 0.4 m/s
Headspace gassing (Air)	0.05 lpm
Dissolved oxygen (DO)	40%
pH for growth	≤ 7.25 controlled by CO ₂
pH for production	6.80 ± 0.05

Transfect cells

1. Subculture and expand cells until they reach a density of $3.5\text{--}5.5 \times 10^6$ viable cells/mL in a culture volume that will yield at least 1×10^9 viable cells to inoculate one bioreactor.
2. Allow cells to recover for at least three passages following thaw before transfecting.
3. Calibrate DO and pH probes and add to assembled bioreactor.
4. Sterilize bioreactor and allow to cool to room temperature.
5. Add 700 mL CTS™ LV-MAX™ Production Medium to the bioreactor. Turn on agitation, headspace gassing, and temperature control. Allow medium to equilibrate to temperature set point before seeding cells.
6. Seed bioreactor by adding cells from Step 1 to a final cell density of 1×10^6 viable cells/mL in a total volume of 1 L.
7. Check calibration of pH and perform a 1 point calibration offset if required.

Transfect cells (Day 0)

8. On the day of transfection, determine viable cell density and percent viability. Cells should have reached a density of approximately $3.5\text{--}5.0 \times 10^6$ cells/mL. Viability should be ≥99% to proceed with transfection.
9. Add pre-warmed CTS™ LV-MAX™ Production Medium to the bioreactor to dilute the cells to a final density of 4.0×10^6 viable cells/mL.

10. Shift pH set point down to 6.8 ± 0.05 controlled by CO₂. Allow bioreactor to equilibrate.
11. Prepare CTS™ LV-MAX™ Transfection Reagent/plasmid DNA complexes.

Note: The final concentration of 2.5 mg plasmid DNA per liter of culture to transfect contains both packaging plasmid mixture and transfer plasmid. At a 3:2 ratio, this equates to 1.5 mg packaging plasmid mixture and 1.0 mg transfer plasmid.

As an option, the amount of packaging plasmid and transfer plasmid can be halved, but the change should be tested to determine the effect on transfection efficiency.

- a. To a 250 mL PETG Nalgene bottle, add Opti-MEM I Medium at 5% of the cell culture volume to be transfected (i.e., 80 mL CTS Opti-MEM I Medium for transfecting 1.6 L of culture).
 - b. Gently invert the bottle of CTS™ LV-MAX™ Transfection Reagent 4–5 times to mix.
 - c. Add CTS™ LV-MAX™ Transfection Reagent to a final concentration of 6 mL/L of culture to be transfected to the bottle in step 11a (i.e., 9.6 mL CTS™ LV-MAX™ Transfection Reagent to transfect 1.6 L of cells).
 - d. To a second 250 mL PETG Nalgene bottle, add Opti-MEM I Medium at 5% of the cell culture volume to be transfected (i.e., 80 mL CTS Opti-MEM I Medium for transfecting 1.6 L of culture).
 - e. Add plasmid DNA to a final concentration of 2.5 mg/L of culture to be transfected to the second 250 mL PETG Nalgene bottle in step 11d (i.e., 4 mg DNA to transfect 1.6 L of cells).
 - f. Combine the two bottles by adding the diluted plasmid DNA (from 11e) to the diluted CTS™ LV-MAX™ Transfection Reagent (from 11c).
12. Incubate CTS™ LV-MAX™ Transfection Reagent/plasmid DNA (from step 11f) at room temperature for 10 minutes, then attach a transfer cap to the bottle, sterile tube weld the bottle to the bioreactor and use a peristaltic pump to transfer the solution into the bioreactor.

Note: For optimal performance, the CTS™ LV-MAX™ Transfection Reagent/plasmid DNA complex should be fully added to the bioreactor within 30 minutes of initiating complexation.

- a. Add CTS™ LV-MAX™ Enhancer at a final volume of 4% v/v (40 mL/L) of culture volume transfected (i.e., 64 mL CTS™ LV-MAX™ Enhancer if transfecting 1.6 L of cells).
 - b. Add CTS™ LV-MAX™ Supplement at a final volume of 5% (50 mL/L) of culture volume transfected (i.e., 80 mL CTS™ LV-MAX™ Supplement if transfecting 1.6 L of cells).
14. Sample Bioreactor daily.
 - a. Count cells on a cell counter.
 - b. Check pH, O₂, and CO₂ on a gas analyzer.
 - c. Check metabolites on a bioanalyzer.

- d. Aseptically remove samples for analysis to determine optimal harvest time, approximately 48 hours post transfection.



Titer lentiviral vector

IMPORTANT! Handling of lentivirus must be performed as per institutional guidelines. All materials should be treated with a 10% bleach solution prior to disposal.

Titer using GFP expression

Procedural guidelines

- Thaw the virus on ice before use. Do not use any acceleration method because it can decrease the virus titer.
- Mix the virus by tapping or inverting the tube. Do not vortex and avoid mixing vigorously.

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.

Item	Source
HT1080 cell line	ATCC, CCL-121
DMEM, high glucose, GlutaMAX™ Supplement, pyruvate ^[1]	10569010
Polybrene ^[2]	Fisher Scientific™, NC0663391
96-well culture plates	MLS
96-well round bottom plates	MLS
Large swinging bucket centrifuge	MLS
TrypLE™ Express Enzyme (1X), no phenol red	12604013
DPBS, no calcium, no magnesium	14190250

^[1] Add 10% FBS to make culture medium for HT1080

^[2] Stock solution: 10 mg/mL in sterile H₂O

Perform lentiviral vector titration

Day 1 (morning) - infect HT1080 cells

1. Four hours before infection, seed a 96-well culture plate according to the following table:

Cells	Density	Volume of media
HT1080	7,000 cells/well	100 μ L/well

Note: Cells are ~30% confluent at time of infection.

2. Two hours before infection, thaw the virus on ice.

Note: Do not accelerate the lentiviral vector thawing process because it can decrease the titer of the virus.

3. Prepare dilution medium by combining 15 mL of fresh culture medium with 12 μ L of 10 mg/mL Polybrene (final concentration of 8 μ g/mL), then vortex to mix.
4. Prepare a 4-log serial dilution (10^{-1} to 10^{-4}) by sequentially diluting 15 μ L of virus into 135 μ L of dilution medium per well of a round bottom 96-well plate.

Note: We recommend performing each dilution in quadruplicate.

Note: If your virus was concentrated, you may need more dilutions.

5. Remove the culture media from the HT1080 cells, then infect by transferring 100 μ L of the diluted virus to each corresponding well.
6. Centrifuge the infected cell plate at $900 \times g$ for 30 minutes at room temperature.
7. Incubate the infected cell plate overnight.

Day 2 (morning) – change medium, then incubate cells

1. Remove the medium from each well, then add 100 μ L/well of fresh HT1080 culture medium (without Polybrene).

Note: When replacing medium, always start from the Negative Control and work backwards to minimize the amount of virus carried over.

2. Incubate the infected plate for 72 hours.

Day 5 - prepare cells, then run through a flow cytometer

1. Remove the medium from each well, then add 150 μ L of trypsinization medium (75% TrypLE™ + 25% DPBS mixture) to each well.
2. Incubate the infected plate for 15 minutes in an incubator at 37°C.
3. Use a microscope to check the cells and ensure that the cells are rounded up.
4. Detach the cells from the culture plate bottom by gently pipetting up and down.
5. Process the cells through a flow cytometer.

Calculate the lentiviral titer

1. To calculate the titer (Transforming Units/mL), determine the appropriate dilution factor to use based on the % of GFP⁺ cells.

The optimal infection range is 1–20% of GFP⁺ cells. Over 20% of GFP⁺ cells results in an underestimated lentiviral vector titer.

Lentivirus dilution	EmGFP ⁺ cells
10 ²	96%
10 ³	65%
10 ⁴	18%

Using this table of results, the 10⁴ dilution is selected for the calculation because the % of GFP⁺ cells falls in the desired 1–20% range.

2. Use the following formula to calculate the titer:

$$\text{Titer} = (F \times C/V) \times D$$

- F = frequency of GFP⁺ cells (%GFP⁺ cells/100)
- C = cell number per well at the time of transduction (7,000 cells)
- V = volume of inoculum in mL (0.1 mL)
- D = lentivirus dilution factor

Example:

- F = 18/100 (using the table in Step 1)
- C = 7,000 (cell number at the time of infection)
- V = 0.1 (100 μ L of medium)
- D = 10⁴

The calculation is:

$$\text{Titer} = (0.18 \times 7,000/0.1) \times 10^4 = 1.26 \times 10^8 \text{ TU/mL}$$

Titer using antibiotic selection

Procedural guidelines

- Thaw the virus on ice before use. Do not use any acceleration method because it can decrease the virus titer.
- Mix the virus by tapping or inverting the tube. Do not vortex and avoid mixing vigorously.

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.

Item	Source
HT1080 cell line	ATCC, CCL-121
DMEM, high glucose, GlutaMAX™ Supplement, pyruvate ^[1]	10569010
Polybrene ^[2]	Fisher Scientific™, NC0663391
Crystal violet	Fisher Scientific™, ICN15251150
Blasticidin S HCl ^[3]	A1113903
Puomycin ^[4]	A1113802
6-well culture plates	140675
TrypLE™ Express Enzyme (1X), no phenol red	12604013

^[1] Add 10% FBS to make culture medium for HT1080

^[2] Stock solution: 10 mg/mL in sterile H₂O and filter through 0.2 µm filter

^[3] Selection medium: culture medium with Blasticidin S HCl, 10 µg/mL final concentration (the selection medium based on pLenti transfer plasmid backbone selection marker)

^[4] Select culture medium final concentration 10 µg/mL

Perform lentiviral vector titration

Day 1 (morning) – infect HT1080 cells

1. Four hours before infecting cells, seed a 6-well plate according to the following table:

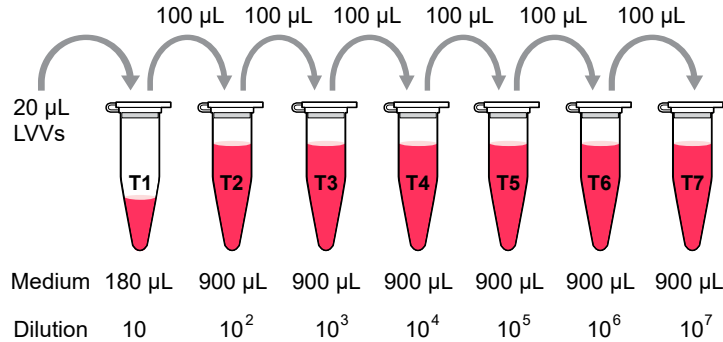
Cells	Density	Volume of media
HT1080	210,000 cells/well	2 mL/well

Note: Cells are ~30% confluent at time of infection.

2. Two hours before infection, thaw the virus on ice.

Note: Do not accelerate the lentiviral vector thawing process because it can decrease the titer of the virus.

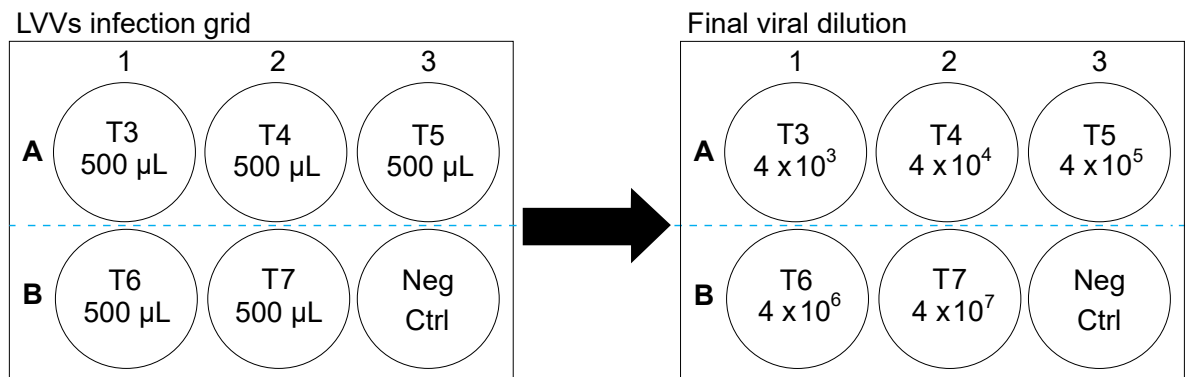
- Prepare dilution medium by combining 20 mL of fresh culture medium with 16 μL of 10 mg/mL Polybrene (final concentration of 8 $\mu\text{g}/\text{mL}$), then vortex to mix.
- For a virus sample, prepare seven microcentrifuge tubes and label them as T1, T2 to T7.
- Add 180 μL of prepared dilution medium to T1, then add 900 μL to T2 through T7.
- Add 20 μL of virus sample, crude lentivirus, or concentrated virus aliquot to T1 (10^1 dilution), then invert the tube to mix.



- Use a new tip to transfer 100 μL of T1 dilution to T2, then invert the tube to mix (10^2 dilution).
- Use a new tip to transfer 100 μL of T2 dilution to T3, then invert the tube to mix (10^3 dilution).
- Repeat Step 8 to complete dilutions T4 to T7.

Note: If your virus was concentrated, you may need more dilutions.

- Replace the culture medium in A1, A2, A3, B1, and B2 of the prepared 6-well plate of HT1080 cells with 1,500 μL of prepared dilution medium, then 2,000 μL to B3 as a negative control ('Neg Ctrl')
- Pipet 500 μL of diluted virus into each respective well, starting with T3, according to the following figure.



- Ensure even distribution of the diluted virus across each well by moving the infected 6-well plate left, right, backward, then forward several times.

13. Centrifuge the infected cell plate at $900 \times g$ (using a swinging bucket centrifuge) at room temperature for 30 minutes.
14. Incubate the infected cell plate overnight.

Day 2 (morning) – change medium, then incubate cells

1. Remove the medium from each well, then add 2 mL/well of fresh HT1080 culture medium (without Polybrene).

Note: When replacing medium, always start from the Negative Control and work backwards to minimize the amount of virus carried over.

2. Incubate cells for 24 hours.

Day 4 – start antibiotic selection

1. Prepare HT1080 culture medium with antibiotic, such as Blasticidin with a final concentration of 10 $\mu\text{g}/\text{mL}$ or Puromycin with a final concentration of 1 $\mu\text{g}/\text{mL}$.

Note: Select antibiotic based on your pLenti-transfer backbone selection marker.

2. Replace the culture medium in each well with 2 mL of prepared selection medium, then incubate for 48 hours.
3. Repeat Step 2 until the 'NegCtrl' well has no cells left.
This is an approximately 10-day process.

Day 13 – stain with Crystal Violet

1. Prepare the Crystal Violet staining solution: 1% Crystal Violet dissolved in a 10% ethanol/ H_2O solution.
2. Remove the antibiotic selection medium from the 6-well plate, then wash each well with 2 mL of PBS.
3. Add 1 mL of prepared Crystal Violet staining solution to each well, then incubate the plate for 20 minutes at room temperature.
4. After 20-minutes incubation, remove the staining solution from the 6-well plate.
Store the staining solution because it can be reused.
5. Wash the stained 6-well plate with water several times until a clear background appears.

Calculate the lentiviral titer

1. Visually count the number of stained colonies per well.
2. Use the following formula to calculate the titer (Transforming Units/mL) of the virus stock:

Titer = (Number of discrete colonies \times dilution factor) / Volume of inoculum

Example:

Count = 30 colonies stained in the 4×10^6 dilution well (B-1) and 5 colonies stained in the 4×10^7 dilution well (B-2)

Volume of diluted virus = 2 mL

The calculation is:

$$\text{Titer} = (30 \times 4 \times 10^6 + 5 \times 4 \times 10^7) / 2 / 2 = 8 \times 10^7 \text{ TU/mL}$$



Scaling up tables

Tables for scaling up preset and custom sized production volumes

This section contains two tables for use in scaling up the production volume.

The first table (Table 11 on page 31) displays all the required volumes for each reagent based on the preset production vessel sizes that can be used.

The second table (Table 12 on page 32) displays the formula for each of the required volumes for each reagent based on the custom production volume to be used. Use the formula in the table for each reagent to calculate your specific volume, then input that volume in the space provided under the correct heading.

An explanation of the scaling up table headings are in the following list:

- **Production vessel:** The vessel used for culturing the lentiviral vector (ensure the vessel size is large enough for the production volume plus all other reagents).
- **Production volume:** This is the initial volume of media and cells combined.
- **Number of cells:** The number of cells required.
- **Volume of media:** The amount of media to dilute the cells in.
- **Supplement:** The volume of CTS™ LV-MAX™ Supplement required.
- **Media (DNA):** The volume of CTS™ Opti-MEM™ I required for tube 1 (DNA).
- **Packaging:** The amount of packaging plasmid required for tube 1 (DNA).
- **Transfer:** The amount of transfer plasmid required for tube 1 (DNA).
- **Media (TfxR):** The volume of CTS™ Opti-MEM™ I required for tube 2 (TfxR).
- **Transfection reagent :** The amount of CTS™ LV-MAX™ Transfection Reagent required for tube 2 (TfxR).
- **Enhancer:** The amount of CTS™ LV-MAX™ Enhancer required.

Table 11 Preset scaling up

Production vessel	Production volume	Diluted cell preparation			Tube 1 (DNA)			Tube 2 (TfxR)		Enhancer
		Number of cells	Volume of media	Supplement	Media (DNA)	Plasmids ^[1]		Media (TfxR)	Transfection reagent	
						Packaging	Transfer			
96 deep well plate	1 mL	4 × 10 ⁶	0.85 mL ^[2]	50 µL	50 µL	1.5 µg	1 µg	50 µL	6 µL	40 µL
50-mL conical	10 mL	4 × 10 ⁷	8.5 mL ^[2]	500 µL	500 µL	15 µg	10 µg	500 µL	60 µL	400 µL
125-mL shaker flask	30 mL	1.2 × 10 ⁸	25.5 mL ^[2]	1.5 mL	1.5 mL	45 µg	30 µg	1.5 mL	180 µL	1.2 mL
1-Liter shaker flask	400 mL	1.6 × 10 ⁹	340 mL ^[2]	20 mL	20 mL	600 µg	400 µg	20 mL	2.4 mL	16 mL
2-Liter shaker flask	1 L	4 × 10 ⁹	850 mL ^[2]	50 mL	50 mL	1.5 mg	1 mg	50 mL	6 mL	40 mL

^[1] The ratio of packaging plasmid to transfer plasmid is 3:2. The final concentration of packaging plasmid is 2.5 µg/mL of production volume.

^[2] Volume of 3-day cultured cells.

Table 12 Custom scale up

Production volume	Diluted cell preparation			Tube 1 (DNA)			Tube 2 (TfxR)		Enhancer
	Number of cells	Volume of media	Supplement	Media (DNA)	Plasmids ^[1]		Media (TfxR)	Transfection reagent	
					Packaging	Transfer			
1 mL	4×10^6	0.85 mL ^[2]	50 μ L	50 μ L	1.5 μ g	1 μ g	50 μ L	6 μ L	40 μ L
n mL	$n \times$ number of cells	$n \times$ Volume of media	$n \times$ volume of Supplement	$n \times$ volume of Media (DNA)	$n \times$ amount of Packaging	$n \times$ amount of Transfer	$n \times$ volume of Media (TfxR)	$n \times$ volume of Transfection reagent	$n \times$ volume of Enhancer
$n \times 1$ mL	$n \times 4 \times 10^6$	$n \times (0.85 \text{ mL } ^{[2]})$	$n \times 50 \mu\text{L}$	$n \times 50 \mu\text{L}$	$n \times 1.5 \mu\text{g}$	$n \times 1 \mu\text{g}$	$n \times 50 \mu\text{L}$	$n \times 6 \mu\text{L}$	$n \times 40 \mu\text{L}$

^[1] The ratio of packaging plasmid to transfer plasmid is 3:2. The final concentration of packaging plasmid is 2.5 μ g/mL of production volume.

^[2] Volume of 3-day cultured cells.



Related products

Related products

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Item	Source
Vivid Colors™ pLenti6.3/V5-GW/EmGFP Expression Control Vector	V37006
Countess™ II Automated Cell Counter	AMQAX1000
Large capacity cell culture incubator controls 37°C, 8% CO ₂ and 75–80% humidity	3950 (if applicable for scale-up)



Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.



Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.



Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf>
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf



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 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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