



USER GUIDE

SHIPPED ON DRY ICE

STORE AT -80°C

RESEARCH USE ONLY

01 Product Description

HiTE™ (High-efficiency Transduction Enhancer) is a next-generation transduction enhancer for use with lentiviral vectors and mammalian cells, including primary T cells, NK cells, iPSCs, and standard cell lines. HiTE™ actively binds both lentivirus and target cells, facilitating viral entry through the membrane while promoting proximity for more efficient gene transfer.

MECHANISM

HiTE™ is a bi-functional Transient Fusion-Promoting Peptide (TFP) that concentrates viral particles at the cell surface and transiently destabilizes the membrane to enhance fusion. The peptide self-inactivates within hours, preserving cell viability and function. See HiTE™ White Paper for complete mechanism and performance data.

02 Reagent & Storage

HiTE™ is supplied at a 10× stock concentration of 400 µM. Store at -80°C for up to 6 months. Thaw on ice and aliquot after first use; up to three freeze/thaw cycles are permitted.

Parameter	Specification	Notes
Stock Concentration	10x - 400 µM	10× concentrated; use 1:10 dilution
Storage Temperature	-80°C	Up to 6 months
Freeze/Thaw Cycles	3 maximum	Aliquot after first thaw
Thawing	On ice	Do not heat or vortex

03 Transduction Protocol

3.1 Recommended Working Concentrations

- Hard-to-transduce suspension cells (T cells, NK cells): 80 μM final (1:5 dilution)
- Standard suspension or adherent cell types: 40 μM final (1:10 dilution)
- iPSCs: 20–40 μM final (1:10 to 1:20 dilution)
- Perform a full media change at 72 hours post-transduction to remove HiTE™.

Quick Reference Table

Cell Type	Plate	Total Vol.	Target Conc.	Dilution	HiTE™ Vol.
Adherent	96-well	100 μL	40 μM	1:10	10 μL
	24-well	500 μL	40 μM	1:10	50 μL
Suspension	96-well	100 μL	80 μM	1:5	20 μL
	24-well	500 μL	80 μM	1:5	100 μL

3.2 Working Protocol — Suspension Cells (96-well)

1. Resuspend 1×10^4 cells in 25 μL of complete medium per well.
2. Add lentivirus at optimized MOI (typically 5–20) in 25 μL of complete medium.
3. Thaw HiTE™ on ice. Add to reach 80 μM final concentration in 50 μL of complete medium.
4. Mix gently and incubate overnight at 37°C with 5% CO_2 .
5. Day 3: Perform complete media change. Proceed with downstream applications.

3.3 Working Protocol — Adherent Cells (96-well)

6. Seed 2×10^4 cells per well in 25 μL of complete medium.
7. Add lentivirus at optimized MOI (typically 2–10) in 25 μL of complete medium.
8. Add HiTE™ to 40 μM final (or 80 μM for difficult lines) in 50 μL of complete medium.
9. Mix gently and incubate overnight at 37°C with 5% CO_2 .
10. Day 3: Perform complete media change. Proceed with downstream applications.

3.4 Working Protocol — 24-well Format

Scale up proportionally: 5×10^5 cells (suspension) or 1×10^5 cells (adherent) in 500 μL total volume. All other steps identical.

04 Optional: Spinfection

HiTE™ does not require spinfection. If desired for particularly difficult cells (e.g., NK cells), centrifuge plates at 800–1000 ×g for 60–90 minutes at 32°C before incubation.

05 Troubleshooting

Issue	Possible Cause	Solution
Low transduction efficiency	MOI too low	Increase MOI (up to 50)
	HiTE™ concentration too low	Increase to 160 µM (1:2.5 dilution)
	Hard-to-transduce cells	Add spinfection step (800×g, 60 min, 32°C)
Low viability	MOI too high	Reduce MOI
	HiTE™ concentration too high	Reduce to 20–40 µM
	Cells not healthy pre-transduction	Ensure >90% viability before transduction
Inconsistent results	HiTE™ degraded	Check storage; avoid >3 freeze/thaw cycles
	Virus quality variable	Titer virus; use fresh aliquots

06 Technical Specifications

Parameter	Specification
Product Type	Transient Fusion-Promoting Peptide (TFP)
Stock Concentration	400 µM in sterile water
Working Concentration	20–160 µM (cell-type dependent)
Molecular Weight	~2,000–6,000 Da
Storage	–80°C, up to 6 months
Shelf Life	>12 months at –80°C
Compatibility	Lentiviral vectors (VSV-G pseudotyped); all mammalian cell types
Regulatory Status	For Research Use Only

For detailed performance data, mechanism of action, and comparative analysis, refer to the HiTE™ White Paper available at www.hitebio.com.