T CELL TRANSDUCTION PROTOCOL

SHIPPED ON DRY ICE	STORE AT −80°C	RESEARCH USE ONLY

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01 Overview & Intended Use

This protocol describes the optimized procedure for using HiTE™ (High-efficiency Transduction Enhancer) to achieve efficient lentiviral transduction of primary human $CD3^+$ T cells for applications including:

- CAR-T cell generation (CD19, BCMA, and other targets)
- TCR-T cell engineering
- Reporter gene expression (GFP, mCherry, luciferase)
- Gene editing delivery (Cas9, base editors)
- Functional genomics screens

HiTE™ is a bi-functional Transient Fusion-Promoting Peptide (TFP) that enhances lentiviral transduction through a dual-binding mechanism. It binds to both lentiviral envelope proteins and target cell membrane receptors, promoting virus-cell proximity and membrane fusion while self-inactivating within hours.

PERFORMANCE NOTE

For detailed performance data and comparative analysis, refer to the HiTE™ White Paper (www.hitebio.com).

02 Safety Information

⚠ BIOSAFETY NOTICE

This protocol involves replication-incompetent lentiviral vectors. All work must be performed in accordance with institutional biosafety guidelines, typically BSL-2 with BSL-2+ practices. Obtain appropriate IBC approval before beginning.

2.1 Personal Protective Equipment

- Laboratory coat (disposable preferred when handling virus)
- Double nitrile gloves
- Safety glasses or face shield
- Closed-toe shoes

2.2 Work Area Requirements

- Certified Class II Type A2 biosafety cabinet (BSC)
- Dedicated incubator for transduced cells (if possible)
- 10% bleach solution for decontamination
- Biohazard waste containers for all virus-contacting materials

HiTE™ is classified as a research reagent with no known hazards at recommended concentrations. Standard laboratory practices apply. Refer to the HiTE™ Safety Data Sheet (SDS) for complete information.

03 Materials Required

Reagent	Catalog #	Storage
HiTE™ (10x, 400 µM stock)	HiTE-RUO-001	−80°C
Lentiviral vector (your construct)	User-supplied	−80°C
Anti-CD3/CD28 beads (e.g., Dynabeads)	Thermo 11161D	4°C
T cell medium (ImmunoCult™-XF or X-Vivo™ 15 recommended)	Various	4°C
Human recombinant IL-2 (50–100 IU/mL)	Various	−20°C
DPBS (Ca^{2+}/Mg^{2+}-free)	Various	RT
Trypan blue or viability dye	Various	RT

04 Reagent Preparation

4.1 HiTE™ Aliquoting (First Use)

1. Thaw HiTE™ vial on ice (5–10 minutes).
2. Gently mix by pipetting. Do not vortex.
3. Aliquot into sterile microcentrifuge tubes (10–20 µL per aliquot recommended).
4. Store aliquots at −80°C. Up to 3 freeze/thaw cycles permitted.

4.2 MOI Calculation

MOI = (Viral titer [TU/mL] × Volume [mL]) / Number of cells

Example:

For MOI 5 with 1×10^5 cells and virus at 1×10^8 TU/mL: Volume = $(5 \times 1\times10^5) / (1\times10^8)$ = 5 µL

05 T Cell Activation (Day −3)

CRITICAL STEP

T cells must be activated 48–72 hours before transduction. Activated T cells transduce significantly more efficiently than resting cells.

1. Thaw or isolate primary human $CD3^+$ T cells. Count and assess viability (>90% required).
2. Resuspend at 1×10^6 cells/mL in complete T cell medium with IL-2 (50–100 IU/mL).
3. Add anti-CD3/CD28 beads at a 1:1 bead-to-cell ratio.
4. Incubate at 37°C, 5% CO_2 for 48–72 hours.

Expected results: >80% CD25+ expression, >70% CD69+ expression, 1.5–2× size increase.

06 Transduction (Day 0)

This is the core protocol step. All work should be performed in a BSC.

6.1 Standard Protocol (24-well plate)

1. Remove beads from activated T cells using a magnetic separator.
2. Count cells and adjust to 5×10^5 cells per well in 200 µL complete medium.
3. Thaw HiTE™ aliquot on ice.
4. Add HiTE™ to achieve 80 µM final concentration (1:5 dilution; 100 µL of 400 µM stock per 500 µL total).
5. Add lentiviral vector at desired MOI (typically MOI 5–10) in remaining volume.
6. Bring total volume to 500 µL with complete medium.
7. Mix gently by pipetting 2–3 times. Do not vortex.
8. Incubate at 37°C, 5% CO_2 for 12–18 hours (overnight).

6.2 HiTE™ Concentration Guide for T Cells

Scenario	Recommended Conc.	Notes
Standard T cell transduction	**80 µM (1:5 dilution)**	Recommended starting point
Maximum efficiency needed	160 µM (1:2.5 dilution)	For hard-to-transduce donors
Viability-sensitive applications	40 µM (1:10 dilution)	Lower efficiency, higher viability

07 Post-Transduction Culture (Day 1–2)

1. Day 1: Observe cells under microscope. Healthy transduced cells should show normal morphology.
2. Add 250 µL fresh complete medium with IL-2 to each well (do not remove existing medium).
3. Day 2: Monitor cell density. If cells are dense (>2×10^6/mL), split 1:2 with fresh medium.

08 Media Change & Analysis (Day 3)

1. Perform complete media change: Centrifuge cells (300×g, 5 min), remove supernatant, resuspend in fresh complete medium.
2. Assess viability by trypan blue exclusion or flow cytometry (PI/Annexin V).
3. Assess transduction efficiency by flow cytometry for reporter gene (GFP, mCherry) or CAR expression.
4. Record cell count, viability, and %transgene-positive cells.

Expected viability: >90%. Expected transduction efficiency: See HiTE™ White Paper for cell-type-specific benchmarks.

09 Extended Culture & Expansion (Day 4+)

For applications requiring expanded T cell populations:

1. Maintain cells at 0.5–1×10⁶ cells/mL in complete medium with IL-2.
2. Feed every 2–3 days by adding fresh medium or splitting.
3. Expected expansion: 50–100× over 10–14 days post-activation.
4. Cryopreserve or use for functional assays as needed.

10 Quality Control & Expected Results

Parameter	Acceptance Criteria	Method
Cell Viability (Day 3)	**>90%**	Trypan blue / flow cytometry
Transduction Efficiency	See White Paper	Flow cytometry (reporter/CAR)
T Cell Phenotype	CD3+ >90%	Flow cytometry
Expansion (Day 14)	50–100×	Cell counting

11 Troubleshooting Guide

Problem	Possible Cause	Solution
Very low efficiency (<10%)	T cells not properly activated	Verify CD25/CD69 expression; ensure 48–72h activation
	Virus inactive or low titer	Re-titer virus; use fresh aliquot
	HiTE™ degraded	
Low viability (<80%)	MOI too high	Reduce MOI by 50%
	Cells not healthy pre-transduction	Start with >90% viable cells
Variable results	Donor variability	Test multiple donors; optimize MOI per donor
	Inconsistent pipetting	Use calibrated pipettes; mix gently
No transgene expression	Vector construct issue	Verify vector with positive control cell line (e.g., HEK293T)

12 Protocol Variations

12.1 High-Throughput Screening (96-well)

Scale down to 1×10⁴ cells/well in 100 µL total volume. Adjust HiTE™ and virus volumes proportionally. Ideal for MOI optimization and dose-response experiments.

12.2 Large-Scale Manufacturing

For GMP-scale manufacturing, HiTE™ can be added directly to culture bags or bioreactors. No modification to the direct-addition protocol is required. Compatible with Miltenyi CliniMACS Prodigy and Lonza Cocoon platforms.

12.3 Repeat Transduction

For applications requiring very high efficiency, a second transduction can be performed on Day 1 using the same protocol. Add fresh HiTE™ and virus without media change.

For complete performance data and comparative analysis, see the HiTE™ White Paper at www.hitebio.com.

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