iPSC TRANSDUCTION PROTOCOL

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

TABLE OF CONTENTS

01 Overview & Intended Use

This protocol provides comprehensive guidance for using HiTE™ to achieve efficient lentiviral transduction of human induced pluripotent stem cells while preserving pluripotency and differentiation potential. The applications may include:

- Stable transgene expression in iPSC lines
- CAR expression for iPSC-derived immune cell manufacturing
- Reporter gene introduction for lineage tracing
- CRISPR/Cas9 delivery for gene editing
- Safety switch insertion (e.g., iCaspase9)
- Disease modeling with genetic modifications

iPSCs: PRESERVING STEMNESS WHILE ACHIEVING EFFICIENT TRANSDUCTION

Induced pluripotent stem cells present a unique challenge: efficient genetic modification must be balanced with preservation of pluripotency markers and differentiation capacity. HiTE™ achieves this balance with superior transduction efficiency while maintaining stem cell identity. For detailed performance data, see the HiTE™ White Paper.

1.1 Supported iPSC Lines

HiTE™ is compatible with a broad range of iPSC lines including WTC-11 (Coriell Institute), ATCC-ACS-1019 and related lines, patient-derived iPSC lines, and GMP-compatible master cell banks.

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 iPSC Transduction Challenges

3.1 Why iPSCs Are Difficult to Transduce

Challenge	Description
Low Basal Efficiency	iPSCs exhibit 10–50% baseline transduction rates at high MOI. Without enhancers, efficiency often drops below 15%.
Pluripotency Sensitivity	Conventional enhancers like Polybrene can trigger spontaneous differentiation and loss of OCT4/NANOG expression.
Toxicity Concerns	iPSCs are more sensitive to membrane-disrupting agents than differentiated cells.
Clonal Selection Needs	Many applications require single-cell cloning, making high initial efficiency critical.
Differentiation Compatibility	Transduced iPSCs must retain capacity for directed differentiation into target lineages.

POLYBRENE DAMAGES iPSCs

Polybrene causes reduced viability and induces spontaneous differentiation in iPSCs. The compound's membrane destabilization triggers differentiation pathways and compromises stem cell identity. Do not use Polybrene for iPSC transduction.

04 Materials Required

Reagent	Catalog #	Storage
HiTE™ (400 µM stock)	HiTE-RUO-002	−80°C
Lentiviral vector (VSV-G pseudotyped)	User-supplied	−80°C
mTeSR Plus or Essential 8 medium	STEMCELL/Gibco	4°C
Matrigel, Vitronectin, or Laminin-521 (coating)	Various	−20°C
ROCK inhibitor (Y-27632, 10 µM)	Various	−20°C
Accutase or TrypLE (dissociation)	Various	4°C

05 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

06 iPSC Culture Considerations

QUALITY CHECK

Only use iPSCs that meet quality criteria prior to transduction: undifferentiated morphology (compact colonies with defined edges), >90% pluripotency marker expression, and normal karyotype. Cells that do not meet these criteria may yield variable transduction efficiency and are at greater risk of losing pluripotency. We recommend passaging cells at least twice before transduction and using cells below passage 20.

- Colony morphology: Compact, well-defined edges, <5% spontaneous differentiation
- Confluence: 50–70% at time of transduction
- Passage: Use gentle enzymatic dissociation (Accutase) for single-cell suspension
- Coating: Matrigel-coated plates for feeder-free culture

07 Transduction Protocol

iPSC-SPECIFIC NOTE

Use a LOWER HiTE™ concentration (40 µM) than for T cells or NK cells. iPSCs are more sensitive, and lower concentrations provide optimal efficiency with preserved pluripotency.

7.1 Protocol Timeline

Day	Action	Notes
Day -1	Prepare iPSCs; ensure 50–70% confluence by D0	Add ROCK inhibitor when plating
Day 0	Transduction with HiTE™	Single-cell suspension; maintain ROCK inhibitor

Day 1	Media change; remove HiTE™	Replace with fresh iPSC medium (no ROCK inhibitor required)
Day 2	Feed cells	Replace with fresh iPSC medium (no ROCK inhibitor required)
Day 3–5	Analyze transduction; assess morphology	Flow cytometry for efficiency and pluripotency

7.2 Step-by-Step Protocol (24-well)

1. Day −1: Pre-treat iPSC culture with 10 µM ROCK inhibitor (Y-27632) for 1–2 hours.
2. Dissociate iPSCs to single cells using Accutase (37°C, 5 min).
3. Count cells. Plate 1×10^5 cells per Matrigel-coated 24-well in 300 µL iPSC medium + ROCK inhibitor.
4. Incubate at 37°C, 5% CO_2 for 24 hours.
5. D0: Thaw HiTE™ on ice. Add to achieve 40-80 µM final concentration.
6. Add lentivirus at MOI 5–10 in 100 µL medium. Mix gently.
7. Bring total to 500 µL with media containing ROCK inhibitor. Incubate overnight (12–18 hours) at 37°C, 5% CO_2.
8. Day 1: Perform full media change. Replace with fresh iPSC medium (no ROCK inhibitor required).
9. Day 2: Feed with normal iPSC medium (no ROCK inhibitor).
10. Day 3–5: Assess transduction by flow cytometry. Check colony morphology.

7.3 HiTE™ Concentration Guide for iPSCs

Scenario	Concentration	Notes
Standard iPSC transduction	**40 µM (1:10)**	Recommended starting point
Higher efficiency needed	80 µM (1:5)	Monitor morphology closely
Sensitive iPSC lines	20 µM (1:20)	Prioritize viability

08 Pluripotency Assessment

After HiTE™-mediated transduction, verify that iPSCs retain pluripotency markers:

8.1 Flow Cytometry Panel

- OCT4 (target: >85%)
- NANOG (target: >85%)
- SSEA-4 (target: >85%)
- TRA-1-60 (target: >85%)
- Reporter/CAR expression (efficiency readout)
- Viability dye

8.2 Morphological Assessment

- Colonies should be compact with well-defined edges
- Minimal spontaneous differentiation (<5%)
- No morphological abnormalities

8.3 Tri-Lineage Differentiation (Optional)

For critical applications, confirm differentiation capacity by directed differentiation into ectoderm, mesoderm, and endoderm lineages using standard protocols. HiTE™-transduced iPSCs should show equivalent differentiation potential to untransduced controls.

09 Downstream Differentiation

HiTE™-transduced iPSCs should retain differentiation capacity compared to untransduced controls. Key considerations:

- Allow 2–3 passages post-transduction before initiating differentiation
- Confirm stable transgene expression before beginning differentiation protocol
- Monitor transgene expression at key differentiation stages
- For single-cell cloning: pick clones at passage 2–3 post-transduction

10 Application: iPSC-Derived Immune Cells

iPSC-derived NK cells and T cells are a major application for HiTE™-mediated iPSC transduction:

10.1 iPSC-NK Manufacturing

- Transduce iPSCs with CAR construct using HiTE™ protocol above
- Select transduced clones by reporter expression or antibiotic selection
- Differentiate using standard iPSC-to-NK protocol (e.g., Zhu & Bhatt, JoVE 2019)
- Verify CAR expression is maintained through differentiation stages

10.2 iPSC-T Cell Manufacturing

- Same transduction workflow as iPSC-NK above
- Differentiate using OP9-DL1/DL4 or organoid protocol
- Verify TCR or CAR expression in mature T cells

11 Application: Gene Editing (CRISPR)

HiTE™ can be used to deliver CRISPR/Cas9 components via lentiviral vectors:

- All-in-one lentiviral Cas9/sgRNA vectors can be transduced using standard HiTE™ protocol
- Use MOI 5–10 and HiTE™ at 40-80 µM for iPSCs

- Select transduced cells by puromycin resistance or fluorescent reporter
- Validate editing by T7 Endonuclease I assay, Sanger sequencing, or NGS

12 Optimization Strategies

12.1 Improving Efficiency

- Increase MOI to 10–15 (monitor viability)
- Increase HiTE™ to 80 µM (1:5 dilution; monitor pluripotency markers closely)
- Ensure iPSCs are well-maintained and <5% spontaneous differentiation
- Use ROCK inhibitor throughout the transduction period

12.2 Improving Viability

- Reduce HiTE™ concentration to 20–60 µM
- Perform media change at 8 hours instead of 24
- Ensure fresh ROCK inhibitor at time of transduction
- Plate cells at higher density (60-80%)

13 Troubleshooting Guide

Problem	Possible Cause	Solution
Low efficiency	iPSC culture quality poor	Start with high-quality undifferentiated cultures
	MOI too low	Increase to MOI 10–15
Differentiation after transduction	HiTE™ concentration too high	Reduce to 20-40 µM (1:20-1:10)
	ROCK inhibitor not used	Add 10 µM Y-27632 during transduction
Cell death	Dissociation too harsh	Use Accutase, not trypsin. Minimize dissociation time.
Variable clones	Integration site effects	Screen multiple clones; use lower MOI for fewer integrations

13 Appendix: iPSC Media Formulations

mTeSR Plus (STEMCELL Technologies)

Use per manufacturer instructions. Suitable for feeder-free iPSC maintenance on Matrigel.

Essential 8 (Gibco)

Use per manufacturer instructions. Suitable for feeder-free iPSC maintenance on Vitronectin.

ROCK Inhibitor Solution

- Dissolve Y-27632 in sterile water at 10 mM stock concentration
- Store aliquots at −20°C
- Working concentration: 10 µM (1:1000 dilution)

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

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