NK CELL TRANSDUCTION PROTOCOL

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

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01 Overview & Intended Use

This protocol provides detailed recommendations for achieving efficient lentiviral transduction of primary human Natural Killer (NK) cells using HiTE™. NK cells represent one of the most challenging cell types for genetic modification, and this protocol addresses the unique requirements for successful NK cell engineering. Some applications may include:

- CAR-NK cell generation (CD19, CD33, NKG2D, and other targets)
- Cytokine-armored NK cells (IL-15, IL-21 secreting)
- Checkpoint receptor knockdown (NKG2A, KIR)
- Reporter gene studies in NK cells
- iPSC-derived NK cell engineering

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.

NK CELLS: UNIQUE TRANSDUCTION CHALLENGES

Natural Killer cells are notoriously difficult to transduce due to rigid plasma membranes, low receptor expression, and heightened sensitivity to cytotoxic enhancers. Polybrene causes >80% viability loss in NK cells. HiTE™ is specifically optimized to overcome these barriers while preserving NK cell function and cytotoxicity. For detailed performance data, see the HiTE™ White Paper.

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 Why NK Cells Require Special Optimization

3.1 Biological Barriers

Challenge	Description
Rigid Plasma Membrane	NK cells have highly organized membrane structures that resist viral fusion, requiring enhanced membrane destabilization for efficient entry.
Low Receptor Expression	VSV-G receptors (LDL-R) are expressed at lower levels on NK cells, reducing natural viral attachment efficiency.
Active Endosomal Barriers	NK cells possess highly active endosomal compartments that can degrade viral particles before genome release.
Cytotoxic Sensitivity	NK cells are extremely sensitive to membrane-disrupting agents. Polybrene causes >80% cell death.
Activation Dependency	Transduction efficiency varies dramatically based on activation status and expansion phase.

POLYBRENE IS TOXIC TO NK CELLS

Polybrene causes severe cytotoxicity in NK cells with viability dropping to ~17% (vs. >80% baseline). This toxicity eliminates any transduction benefit. Do not use Polybrene for NK cell transduction.

3.2 How HiTE™ Overcomes These Barriers

- Targeted membrane engagement without prolonged destabilization
- Transient action window allows efficient viral entry then self-inactivation
- Compatible with NK-specific cytokine cocktails (IL-2, IL-15)
- Maintains NK cytotoxic function and ADCC capacity

04 Materials Required

Reagent	Catalog #	Storage
HiTE™ (400 µM stock)	HiTE-RUO-002	−80°C
Lentiviral vector (VSV-G pseudotyped)	User-supplied	−80°C
NK MACS Medium or equivalent	Miltenyi 130-114-429	4°C
Human recombinant IL-2 (500 IU/mL)	Various	−20°C
Human recombinant IL-15 (10 ng/mL)	Various	−20°C

4.1 NK Cell Source Comparison

Source	Characteristics	Recommended MOI
Leukopak (primary)	Most clinically relevant; requires isolation and expansion	10–20
Purified NK cells	Pre-isolated; variable activation state	10–20
Cord blood NK	Immature phenotype; requires expansion	10–20
NK-92 cell line	Standardized; easier to transduce; not primary	5–10
iPSC-derived NK	Allogeneic; transduce at iPSC stage preferred	5–10

05 Reagent Preparation

5.1 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.

5.2 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 NK Cell Source and Preparation

6.1 Isolation from PBMCs

1. Isolate PBMCs from Leukopak by Ficoll density gradient centrifugation.
2. Deplete non-NK cells using NK cell isolation kit (negative selection).
3. Assess purity by flow cytometry: Target CD3−CD56+ >90%.
4. Count viable cells by trypan blue exclusion.

6.2 NK-92 Cell Line Handling

Maintain NK-92 cells in Alpha-MEM + 12.5% FBS + 12.5% horse serum + 200 IU/mL IL-2. Passage every 2–3 days at 2–3×10⁵ cells/mL. Ensure >90% viability before transduction.

07 NK Cell Activation and Expansion

CRITICAL

NK cells must be activated and expanded for 5–10 days before transduction. Resting NK cells transduce very poorly. The activation state at time of transduction dramatically impacts efficiency.

7.1 Pre-Transduction Expansion Protocol (Day −3 to Day 0)

1. Day −3: Plate NK cells at 5×10^5 cells/mL in NK medium + IL-2 (500 IU/mL) + IL-15 (10 ng/mL).
2. Day 0: Transduce (see Section 07).

08 Optimized Transduction Protocol

NK cells require specific optimization parameters that differ from T cell protocols:

Parameter	NK-Optimized Setting
HiTE™ concentration	**80-100 µM (1:5 to 1:4 dilution)**
MOI	**10–20**
Spinfection	**Optional: 1000×g, 90 min, 32°C (consider for difficult donors)**
Cell density	5×10^5 cells/well (24-well)
Incubation	Overnight at 37°C, 5% CO_2

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

1. Prepare expanded NK cells: count and assess viability (>90% required).
2. Resuspend 5×10^5 NK cells per well in 200 µL NK medium.
3. Thaw HiTE™ on ice. Add to achieve 80-100 µM final concentration (1:5 to 1:4 dilution of 400 µM stock).
4. Add lentiviral vector at MOI 10–20.
5. Bring total volume to 500 µL with NK medium + IL-2 (500 IU/mL) + IL-15 (10 ng/mL).
6. Mix gently by pipetting.
7. Optional: Spinfect by centrifuging plate at 1000×g for 90 minutes at 32°C.
8. Return plate to incubator at 37°C, 5% CO_2 overnight.
9. Day 1: Add 500 µL fresh NK medium with cytokines (do not remove existing medium).
10. Day 3: Perform full media change. Assess transduction and viability by flow cytometry.

8.2 Experimental Controls

Control	Purpose
Untransduced + cytokines	Baseline viability and expansion
Virus only (no HiTE™)	Baseline transduction without enhancer
HiTE™ only (no virus)	HiTE™ toxicity assessment

09 Post-Transduction Expansion

1. Day 1: Add fresh medium with IL-2 + IL-15. Do not remove transduction medium.
2. Day 3: Full media change. Assess transduction efficiency by flow cytometry.
3. Day 7–14: Continue expansion with cytokine feeding every 2–3 days. Expected expansion: 10–50× by Day 14.

TIP

Fresh IL-2 and IL-15 at every feeding step are critical for NK cell expansion and maintenance of cytotoxic function post-transduction.

10 Analysis and Functional Assessment

10.1 Flow Cytometry Panel

- CD3 (T cell exclusion)
- CD56 (NK cell identity)
- CAR or reporter (transgene expression)
- Viability dye (7-AAD or PI)
- CD16, NKG2D (functional markers, optional)

10.2 NK-Specific Gating Strategy

Gate on live cells → singlets → CD3−CD56+ → assess CAR/reporter expression within this population.

10.3 Functional Assays

- Cytotoxicity assay: Co-culture with target cells at E:T ratios of 1:1, 5:1, 10:1
- Cytokine secretion: IFN-γ, TNF-α by intracellular staining or ELISA
- Degranulation: CD107a surface staining after target cell stimulation

11 Optimization Strategies

11.1 Improving Transduction Efficiency

- Increase MOI (up to 30 for primary NK cells)
- Increase HiTE™ concentration to 120–160 µM
- Use spinfection (optional; consider for difficult donors or low-efficiency donors)
- Repeat transduction on Day 1 with fresh HiTE™ + virus
- Optimize activation timing (5–7 days post-isolation is typically best)

11.2 Improving Viability

- Reduce HiTE™ concentration to 40–60 µM
- Perform earlier media change (24 hours instead of 72)
- Ensure fresh cytokines (IL-2 + IL-15) at every step
- Use lower MOI if viability is compromised

12 Troubleshooting Guide

Problem	Possible Cause	Solution
Very low efficiency	No spinfection performed	Add spinfection (1000×g, 90 min, 32°C)
	MOI too low for NK cells	Increase to MOI 20–30
Low viability	HiTE™ concentration too high	Reduce to 40–60 µM
	Poor NK cell health pre-transduction	Ensure >90% viability; check cytokine activity
CAR expression drops	Silencing over time	Use EF1α or PGK promoter (more resistant to silencing in NK)
Donor variability	Normal biological variation	Screen multiple donors; optimize MOI per donor

13 Appendix: NK Cell Media Formulations

NK Expansion Medium

- NK MACS Medium (Miltenyi) or CTS NK Xpander Medium (Gibco)
- 500 IU/mL recombinant human IL-2
- 10 ng/mL recombinant human IL-15
- 1% Pen/Strep (optional)

NK-92 Culture Medium

- Alpha-MEM
- 12.5% FBS
- 12.5% horse serum
- 200 IU/mL recombinant human IL-2
- 1% Pen/Strep

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

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