Lentivirus Transduction Protocol

Standard Transduction Protocol (Adherent Cells)

This protocol serves as a general guideline and should be optimized for each target cell type.

1. Seed target cells in a 24-well plate at 5 x 10^4 cells/well in 0.5 ml complete growth medium. Incubate at 37°C with 5% CO_2 overnight.
   •Note: Adjust cell number and volume proportionally for other plate formats. Include additional wells for a GFP positive control virus, insert-empty negative control virus, and uninfected cells.
2. Prepare complete medium containing lentivirus at the desired volume and MOI.
3. Remove culture medium and replace with 0.5 ml of virus mixture per well.
   •Optional: We recommend adding ViralEntry™ Transduction Enhancer (Cat. No. G515) at a ratio of 1:100 to the culture medium when working with difficult-to-transduce cells or transductions that require a MOI greater than 10.
4. Incubate cells with virus at 37°C with 5% CO_2 overnight.
5. Replace viral supernatant with fresh complete medium and incubate for an additional 48 h.
6. Subculture the cells to achieve 10-30% confluence in a new dish and apply antibiotic selection at the minimum effective concentration determined by kill curve, if required.
7. Assay downstream expression by appropriate methods (e.g. flow cytometry, Western blot, RT-qPCR).

Spinoculation Protocol (Suspension Cells)

Volumes are provided for a 24-well plate format.

1. Grow target cells to confluency.
2. Collect cells by centrifugation at 500 x g for 5 min and aspirate the supernatant.
3. Resuspend cells to a density of 2-5 x 10^5 cells/ml in pre-warmed complete medium. Aliquot 500 µl cells per reaction per 24-well.
4. Add lentivirus at the predetermined MOI. Include additional wells for a GFP positive control virus, insert-empty negative control virus, and uninfected cells.
   •Optional: We recommend adding ViralEntry™ Transduction Enhancer (Cat. No. G515) at a ratio of 1:100 to the culture medium when working with difficult-to-transduce cells or transductions that require a MOI greater than 10.
5. Centrifuge at 1000 x g for 2 h at 30°C.
6. Add complete medium to a final volume of 1 ml and incubate at 37°C with 5% CO_2 for 72 h.
7. Subculture the cells to achieve 10-30% confluence in a new dish and apply antibiotic selection at the minimum effective concentration determined by kill curve, if required.
8. Assay downstream expression by appropriate methods (e.g. flow cytometry, Western blot, RT-qPCR).

Table 1: Key Parameters for Lentivirus Transduction

Parameter	Best Practice Recommendations
Multiplicity of Infection	Multiplicity of infection (MOI) is the ratio of infectious viral particles to target cells and directly impacts transduction efficiency and cell viability. Because optimal MOI varies by cell type, perform pilot transductions with a matched control lentivirus across a range of MOIs (e.g. 0.5-50). Select the lowest MOI that achieves the desired expression while minimizing cytotoxicity.
Transduction Efficiency	Transduction efficiency depends on multiple factors, including viral titer and purity, exposure time, plate format, cell type, passage number, and mycoplasma status. Experimental conditions should match those used during optimization to ensure reproducibility.
Spinoculation	Spinoculation (centrifugation during viral exposure) can enhance lentiviral transduction by increasing virus-cell contact. This approach is particularly useful for difficult-to-transduce or suspension cells (e.g. T cells).
Selection	Antibiotic selection enriches for successfully transduced cells and improves population uniformity. A kill curve should be performed in advance to determine the minimum antibiotic concentration required to eliminate non-transduced cells. Selection typically begins 24-72 h post-transduction and continues until control cells are fully eliminated.
Downstream Assays	Downstream analyses should be performed only after stable gene expression or selection has been achieved. Confirm transgene expression, knockdown or knockout efficiency at the RNA and/or protein level prior to functional assays.

Table 2: Recommended Lentivirus MOIs for Common Cell Lines

Cell Line	Description	Suggested MOI
A431	Human Epidermal Carcinoma	5
A549	Human Lung Carcinoma	5
B-16F10	Mouse Skin Melanoma, Metastatic	5
BxPC3-	Human Pancreatic Adenocarcinoma	10
H3255	Human Non-small Cell Lung Cancer	10
HCT116	Human Colon Carcinoma	5
HeLa	Human Cervical Carcinoma	3
Hepa6-1	Mouse Liver Carcinoma	3
HT29-	Human Colon Adenocarcinoma	3
Jurkat	Human Acute T Cell Leukemia	10
LLC1-	Mouse Lung Carcinoma	6
LNCaP	Human Prostate Carcinoma	5
MM200	Human Skin Melanoma	5
MCF7-	Human Breast Adenocarcinoma	2
MDA-MB231-	Human Breast Adenocarcinoma	1
MM-AN	Human Skin Melanoma, Metastatic	16
MMC	Mouse Breast Carcinoma	4
MRC5-	Human Embryonic Lung Fibroblasts	1
NB4	Human Acute Promyelocytic Leukemia	10
PC12	Rat Adrenal Gland Pheochromocytoma	20
SKOV3-	Human Ovary Adenocarcinoma	15
U2-OS	Human Bone Osteosarcoma	5