Invitrogen™ LentiArray™ CRISPR gRNA Lentivirus

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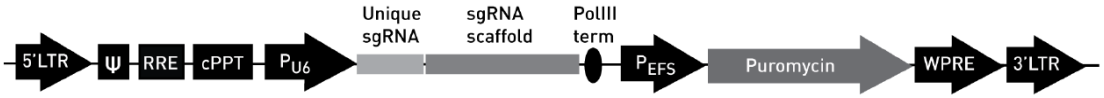
WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Invitrogen™ LentiArray™ CRISPR gRNAs are pre-designed, pre-packaged gRNA lentiviruses designed for efficient gene knockout. LentiArray™ CRISPR gRNA designs have been created with a proprietary gRNA design algorithm and are optimized for maximum knockout efficiency without compromising specificity. LentiArray™ CRISPR gRNAs are available to support pre-screen assay development and post-screen hit validation when performing high-throughput screening experiments with the LentiArray™ CRISPR libraries. LentiArray™ CRISPR gRNA can also be used with LentiArray™ Cas9 lentivirus to perform genome editing experiments in hard-to-transfect cell lines and primary cells.

Characteristic	Description
Product	Invitrogen™ LentiArray™ CRISPR gRNA Lentivirus
Amount	200 µL per gene target
Viral titer	• Libraries are delivered with a range of average titer between 2 × 10^7–2 × 10^8 TU / mL by puromycin antibiotic selection. • We recommend using 1 × 10^8 TU / mL for starting MOI calculations, see section "MOI determination" on page 2 for additional guidance.
Lentiviral map	 • gRNA expression is driven by the U6 promoter. • Expression of the puromycin resistance gene is driven by the EF1 promoter.
Storage	Store at –80°C. Avoid repeated freeze/thaw cycles, which will severely reduce functional viral titer. All components are stable for at least one year after receipt when stored as directed.
Biosafety precaution	LentiArray™ CRISPR gRNA Lentiviruses are packaged using the third generation lentiviral packaging system that has been designed to maximize its biosafety features. Although they are replication-incompetent virions, we recommend treating them as Biosafety Level 2 (BL-2) organisms and following all published BL-2 guidelines with proper waste decontamination procedures.

Procedural guidelines

Getting started

To maximize the performance of the LentiArray™ CRISPR gRNA Lentivirus, determine the growth kinetics, puromycin sensitivity, Polybrene™ tolerance, and transduction efficiency of your cell line.

Choosing cells for transduction

- The LentiArray™ gRNA lentiviruses can be directly delivered into mammalian cells stably expressing Cas9 nuclease or co-infected with Invitrogen™ LentiArray™ Cas9 Lentivirus (Cat. Nos. A32064, A32069) into the target cells. The LentiArray™ Cas9 Lentivirus can also be used to create Cas9 stable cell lines.
- While using a cell line that stably expresses the Cas9 nuclease decreases the variability of CRISPR library screens, co-infection method is advantageous when you do not wish to create a Cas9-stable cell line or you are using cell lines where it may not be possible to isolate a stable clone (such as terminally differentiated stem cells and primary cells).

Transduction conditions

- You must determine the transduction conditions and multiplicity of infection (MOI) for each cell line empirically. If co-infection is needed, we recommend using an MOI ratio of 5–10 for Cas9 to LentiArray™ CRISPR gRNA Lentivirus to achieve the optimal degree of gene knockout.

Note: The nature of your mammalian cell line (e.g., non-dividing vs. dividing cell type) affects the optimal MOI for successful transduction and knockout of the target gene. For example, HT1080 cells are readily transducible, and an MOI of 1 gives transduction efficiencies of around 90%. In some cell types, a 10-fold higher MOI may be needed to get the same transduction efficiency.

- Using culture media containing lower levels of FBS (e.g. 3–5% FBS) during infection may increase the transduction efficiency for some cell types.
- Polybrene™ (hexadimethrine bromide) can enhance the transduction efficiency of lentivirus by 2–10-fold. Before performing any transductions, determine the Polybrene™ tolerance level for your target cells empirically. We recommend testing Polybrene™ within a concentration range of 2–8 µg/mL, then using the highest tolerated concentration (up to 8 µg/mL) in your transductions. While Polybrene™ enhances viral transduction, the use of Polybrene™ is not a requirement for successful transduction. If you observe toxicity or phenotypic changes, titrate down the Polybrene™ concentration or omit it altogether.
- Keep the volume of lentivirus to less than 30–50% of the total volume in the well. If toxicity is observed after transduction, decrease the virus volume (use a lower MOI) and/or omit Polybrene™.
- We have observed that centrifugation at 800 × g for 30–120 minutes at room temperature after the addition of lentivirus to the cells enhances viral infectivity.

- If you plan to use puromycin for selection, you must first determine the optimal antibiotic concentration necessary for the selection of transduced cells. Antibiotic lot, cell type, cell growth kinetics, and cell culture conditions, including cell density, affect the amount of antibiotic that is required for selection. A typical selection with puromycin takes 7–10 days.

MOI determination

- Multiplicity of infection (MOI) is the ratio of the number of virus particles to the number of target cells. The MOI that provides optimal transduction and editing efficiency needs to be determined for each cell line empirically.
- We recommend the use of the LentiArray™ Positive and Negative controls with GFP to identify the MOI for which provides efficient transduction of your cell line. For more information on using the LentiArray™ Positive and Negative controls with GFP, see the LentiArray™ CRISPR Control Lentivirus User Guide (MAN00015949).
- Once you identify the MOI that provides efficient transduction of your cell line utilizing the LentiArray™ controls it is recommended to test a small range of MOI's based on the mean titer of 1×10^8 TU/ml to optimize for the MOI that provides maximum editing efficiency. For example, if an MOI of 2 was found to provide good transduction efficiency (i.e. 90% or greater GFP positive cells) then utilizing your desired assay test an MOI of 1, 2, 4, 8 to determine the MOI that delivers the best editing efficiency and functional results to support downstream workflows.

Methods

Puromycin killing curve

We recommend performing a kill curve with each new cell type or puromycin lot, or with changes to the cell culture conditions to determine the optimal concentration that is needed to eliminate non-transduced cells.

Day 1

Seed cells at the appropriate density in 2 mL/well of complete growth medium in a 6-well culture plate so that the culture is ~80% confluent on the day of antibiotic selection.

Day 2

Remove and replace the medium with 2 mL/well of growth medium containing varying concentrations of puromycin. We recommend testing selection with final puromycin concentrations of 0, 0.5, 1, 1.5, 2.5, 3, 4, 5, and 6 µg/mL.

Day 3–14

Change the selective medium every 3 days for up to 2 weeks, and examine cell viability every 2 days. Select the lowest antibiotic concentration that causes complete cell death after 7 days.

Day 15

After 14 days of selection, the percentage of cells that have survived from puromycin selection can be measured using crystal violet staining or PrestoBlue™ Cell Viability Reagent (Cat. No. A13261).

Suggested transduction protocol in a 96-well plate

The following procedure describes the suggested transduction protocol using the LentiArray™ CRISPR gRNA Lentivirus in a 96-well plate format. You can scale up the procedure based on your experimental needs.

We recommend that you perform the procedure in duplicate or triplicate and include positive and negative controls on each plate. You can perform the experiments using Cas9 stable cells or by co-infecting your cells with LentiArray™ CRISPR gRNA Lentivirus and LentiArray™ Cas9 Lentivirus.

IMPORTANT! Before starting, review the "Procedural guidelines" on page 1.

Day 1

Seed the appropriate number of cells in 100 µL/well of complete growth medium in a 96-well plate to obtain ~50% confluence on the following day. Incubate the cells overnight at 37°C in a humidified 5% CO_2 incubator.

Note: Growth rates vary by cell type and culture condition, and must be determined empirically before starting the screen. When using HT1080 cells, we usually seed 5000 cells in 100 µL/well culture medium in a 96-well plate.

Day 2

1. Remove the tube containing your LentiArray™ CRISPR gRNA Lentivirus and place in a 37°C water bath to thaw. When the tube contents are mostly thawed with only small ice crystals remaining, place the tube on ice.
2. Before opening, centrifuge the lentivirus tube at low speed (maximum RCF at 200 × *g*) for 30 seconds to collect the contents at the bottom of the tube.
3. Replace the spent medium in the wells with complete growth medium containing Polybrene™.

   Note: The optimal Polybrene™ amount varies with cell type and must be determined empirically (see "Procedural guidelines" on page 1).
4. Add the appropriate amount of lentivirus to the cells in the 96-well plate at the suitable multiplicity of infection (MOI) (see "Procedural guidelines" on page 1). Gently swirl the plate to evenly distribute the lentivirus across each well.
5. Incubate the cells overnight at 37°C in a humidified 5% CO_2 incubator.

   Note: Centrifugation at 800 × *g* for 30–120 minutes at room temperature after adding the virus to the cells, also known as *Spinfection*, can enhance the viral infectivity.

Day 3

Remove the media containing lentivirus and replace it with 100 µL/well of complete culture medium.

Day 4

The following steps describe the optional puromycin selection of transduced cells, which takes place from Day 4 to Day 9+. A typical selection with puromycin takes 7–10 days.

Note: Puromycin selection is optional. You can perform the experiment without puromycin selection if you have used high MOI for transduction.

1. Remove the medium containing the lentivirus and add fresh medium containing the appropriate amount of puromycin for the selection of transduced cells (see "Puromycin killing curve" on page 2).
2. Replace the spent medium with fresh medium containing puromycin every 3–4 days.

Day 9+

After selection is complete, perform the desired phenotypic assays (e.g., cell survival, surface protein expression, high-content imaging of cells, reporter assay etc.).

Note: You can isolate single cell clones from the selected pool using serial dilution in 96-well plates as described in the Invitrogen™ LentiArray™ Cas9 Lentivirus user guide (Pub. No. MAN0016088), which is available for download at **thermofisher.com**.

Related products

Product	Cat. No.
Invitrogen™ LentiArray™ Cas9 Lentivirus, 100 µL	A32064
Invitrogen™ LentiArray™ Cas9 Lentivirus, 1 mL	A32069
Invitrogen™ LentiArray™ CRISPR Positive Control Lentivirus, Human HPRT	A32056
Invitrogen™ LentiArray™ CRISPR Positive Control Lentivirus with GFP, Human HPRT	A32060
Invitrogen™ LentiArray™ CRISPR Negative Control Lentivirus, Human Scrambled	A32062
Invitrogen™ LentiArray™ CRISPR Negative Control Lentivirus with GFP, Human Scrambled	A32063
Invitrogen™ LentiArray™ Custom CRISPR Plate	A32045
GeneArt™ Genomic Cleavage Detection Kit	A24372

Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, CA 92008
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.