Invitrogen™ LentiArray™ CRISPR Control Lentivirus Particles

LentiArray™ CRISPR Positive and Negative Control Lentiviruses for use with LentiArray™ Human CRISPR Libraries

Catalog Numbers A32056, A32060, A32062, A32063, A32327, A32829, A32830, and A32831

Pub. No. MAN0015949 **Rev.** B.0

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 Positive Control Lentivirus and Invitrogen™ LentiArray™ CRISPR Negative Control Lentivirus are pre-packaged, ready-to-use lentiviral particles designed as positive and negative controls for experiments using the LentiArray™ CRISPR libraries. The particles are used for the optimization of transduction and antibiotic selection conditions, and can be used as on-plate controls for screening experiments using the LentiArray™ CRISPR libraries.

The positive control particles express gRNAs that target the human hypoxanthine-guanine phosphoribosyltransferase 1 (HPRT1) gene, while the negative control particles express a non-targeting gRNA that does not match any sequence in the human genome. Both the positive and negative control particles express the puromycin resistance gene and are available with or without an EmGFP reporter to provide a visual readout of viral transduction when determining MOI. See Table 1, for the gRNA target sequences.

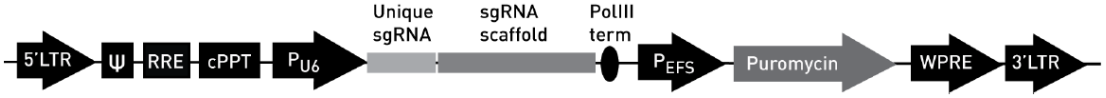
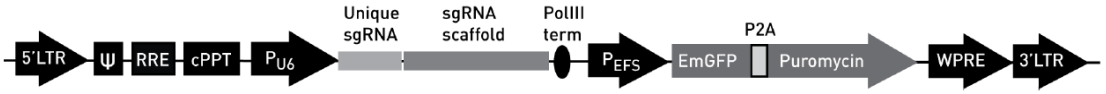
Characteristic	Description
Product	Invitrogen™ LentiArray™ CRISPR Positive Control Lentivirus, Human HPRT (Cat. No. A32056, A32829) Invitrogen™ LentiArray™ CRISPR Positive Control Lentivirus with GFP, Human HPRT (Cat. No. A32060, A32830) Invitrogen™ LentiArray™ CRISPR Negative Control Lentivirus, Human Non-Targeting (Cat. No. A32062, A32327) Invitrogen™ LentiArray™ CRISPR Negative Control Lentivirus with GFP, Human Non-Targeting (Cat. No. A32063, A32831)
Amount	100 µL (Cat. Nos. A32056, A32060, A32062, A32063) 1 mL (Cat. Nos. A32327, A32829, A32830, A32831)
Viral titer	≥1×10^7 TU/mL (transducing unit per mL) by puromycin antibiotic selection (see the Certificate of Analysis for each lot)
Lentiviral map (without EmGFP)	 • gRNA expression (HPRT1 or non-targeting) is driven by the human U6 promoter. • Includes puromycin resistance gene to allow selection of transduced cells.
Lentiviral map (with EmGFP)	 • gRNA expression (HPRT1 or non-targeting) is driven by the human U6 promoter. • Puromycin resistance gene is linked to EmGFP through the self-cleavage P2A peptide.
Storage	Store at –80°C. Avoid repeated freeze/thaw cycles, which can severely reduce functional viral titer. All components are stable for at least one year after receipt when stored as directed.
Biosafety precaution	LentiArray™ CRISPR Control Lentivirus particles have been packaged using a 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 for the use of personal protection equipment and proper waste decontamination procedures.

Table 1 Target sequences for the positive control (HPRT1) and negative control (non-targeting) lentivirus particles.

Control	Target	DNA Target Sequence
LentiArray™ CRISPR Positive Control Lentivirus	HPRT1 gRNA	ATTATGCTGAGGATTTGGAA
LentiArray™ CRISPR Negative Control Lentivirus	Non-targeting gRNA	GTACGTCGGTATAACTCCTC

Procedural guidelines

Strategies for transduction

- The Invitrogen™ LentiArray™ CRISPR Positive and Negative Control Lentivirus particles (with or without GFP) can be used to optimize the transduction and antibiotic selection conditions for your cell line of the interest. We strongly recommend that you include the desired positive and negative controls showing specific phenotype to optimize the assay condition in addition to LentiArray™ CRISPR Positive and Negative Control Lentivirus particles.
- The lentivirus particles in the LentiArray™ Human CRISPR libraries can be delivered into human cells stably expressing Cas9 nuclease or co-infected with Invitrogen™ LentiArray™ Cas9 Lentivirus particles (Cat. Nos. A32064, A32069) into the target cells for screens.
- The advantage of the co-infection approach is that it eliminates the time-consuming process of generating Cas9 stable cell lines. However, using a cell line that stably expresses the Cas9 nuclease decreases the variability of CRISPR library screens. The LentiArray™ Cas9 Lentivirus particles also provide an easy and efficient way to generate cell lines stably expressing Cas9.

MOI determination

- Multiplicity of infection (MOI) is the ratio of the number of virus particles to the number of target cells and must be determined for each cell line empirically.
- The nature of your human 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 ~90%. In some cell types, a 10-fold higher MOI may be needed to get the same transduction efficiency.
- We recommend using the LentiArray™ CRISPR Positive and Negative Control Lentivirus particles with GFP to determine the MOI that allows for maximum editing efficiency.

Transduction conditions

- You must determine the transduction conditions and MOI for each cell line empirically. If co-infection is needed, we recommended using an MOI ratio of 5–10 for Cas9 to CRISPR library lentivirus particles to achieve the optimal degree of gene knockout.
- 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. You must determine the optimal Polybrene™ concentration for your target cells (e.g., maximal infectivity with minimal toxicity) empirically. We recommend initially testing Polybrene™ tolerance with a concentration range (2–8 µg/mL).
- We have observed that centrifugation at 800 × g at room temperature for 30–120 minutes 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 puromycin 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 puromycin that is required for selection. A typical selection with puromycin takes 7–10 days.

Methods

Puromycin kill 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 nontransduced 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, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, and 6 µg/mL.

Day 3–14

1. Change the selective medium every 3 days for up to 2 weeks, then examine cell viability every 2 days.
2. 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).

Determine optimal MOI for transduction

The following procedure describes a general transduction protocol in a 96-well format for testing various MOI to determine the optimal MOI for your cell line using the LentiArray™ CRISPR Control Lentivirus particles.

IMPORTANT! Before starting, review the "Procedural guidelines".

Day 1

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

Note: Growth rates vary by cell type and culture condition, and must be determined empirically. When using HT1080 cells, we usually seed 5,000 cells in 100-µL culture medium per well in a 96-well plate.

Day 2

1. Replace the spent medium in the wells with complete growth medium containing 8 µg/mL of Polybrene™.
2. Remove the tube containing the LentiArray™ CRISPR Control Lentivirus particles and place in a 37°C water bath to thaw. When the viral particles are mostly thawed with only a small ice crystal remaining, transfer the tube onto ice.
3. 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.
4. Prepare a serial dilution of the control lentivirus particles to test a range of MOIs.
5. Add the appropriate volume of control lentivirus from each dilution to the corresponding well in the 96-well plate. Gently swirl the plate to distribute the lentivirus dilutions evenly across each well.

   Note: Centrifuge the cells at 800 × *g* at room temperature for 30 minutes after adding the virus to the cells to enhance the viral infectivity.
6. Incubate the cells at 37°C overnight in a humidified 5% CO_2 incubator.

Day 3

1. To minimize toxicity from virus and polybrene, aspirate virus and add 100 µL/well of complete growth medium post-trasduction at 24 hours.
2. Incubate the cells at 37°C overnight in a humidified 5% CO_2 incubator.

Day 4

If you are using control lentivirus particles with GFP, determine the transduction efficiency by measuring the percentage of GFP expressing cells by fluorescence microscopy (Figure 1) or flow cytometry.

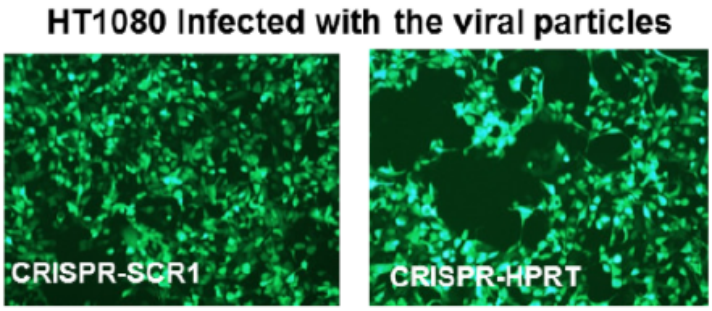


Fig. 1 HT1080 cells were transduced with LentiArray™ CRISPR Negative (left) and Positive (right) Control Lentivirus particles expressing EmGFP and imaged using the Invitrogen™ EVOS™ FL Cell Imaging System. The images show close to 100% transduction efficiency with both controls.

Otherwise, remove the medium containing viral particles and add fresh medium containing the appropriate amount of puromycin for the selection of transduced cells (see "Transduction conditions" on page 2). Replace the spent medium with fresh medium containing puromycin every 3–4 days.

Verify gene editing efficiency

Verify the gene editing efficiency of the control target and select the condition that shows the highest level of editing efficiency in future screening experiments.

We recommend using the GeneArt™ Genomic Cleavage Detection Kit (Cat. No. A24372) to verify the editing efficiency. The genomic cleavage detection (GCD) assay provides a rapid method for evaluating the efficiency of indel formation following an editing experiment.

For more information and protocols, see the GeneArt™ Genomic Cleavage Detection Kit User Guide (MAN0009849), available for download at **thermofisher.com**. To perform the GCD assay for the HPRT1 positive control, you need the primers listed in Table 2.

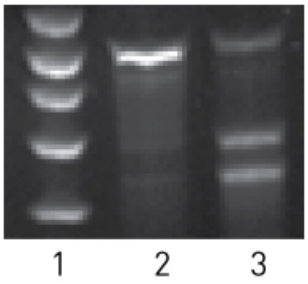


Fig. 2 Cas9 stable U2OS cells were transduced with LentiArray™ CRISPR Lentivirus particles expressing non-targeting (lane 2) or human HPRT1 (lane 3) gRNA sequences. The cells were harvested 2 days post-transduction and analyzed using the GeneArt™ Genomic Cleavage Detection Kit. The transduction using the positive control gRNA (lane 3) shows ~80% cleavage efficiency in the GCD assay.

Table 2 Recommended primer sequences for the GCD assay to verify the gene editing efficiency at the positive control target (HPRT1).

Primer	Sequence
HPRT1 GCD forward primer	TACACGTGTGAACCAACCCG
HPRT1 GCD reverse primer	GTAAGGCCCTCCTCTTTTATTT

Verify functional knock-out

You can verify the functionality of the HPRT1 gene knock-out using the 6-thioguanine (6-TG) resistance assay. Because the cytotoxic mechanism of the anti-cancer chemotherapy drug 6-TG is initiated in cells by HPRT1, HPRT1 knockout cells are resistant to 6-TG treatment.

The following protocol uses cells that have been transduced with the control lentiviral particles and selected with puromycin. The selected cells are then treated with 6-TG for 5 days, and cell survival is evaluated using crystal violet staining or PrestoBlue™ Cell Viability Reagent (Cat. No. A13261).

Day 1

Incubate the transduced cells in 100 µL/well of complete growth medium containing 10 µM and 15 µM of 6-TG at 37°C in a humidified 5% CO_2 incubator for 5 days. Replace the spent medium every other day.

Day 6

Evaluate the percentage of cells that have survived 6-TG selection using crystal violet staining or PrestoBlue™ Cell Viability Reagent (Cat. No. A13261).

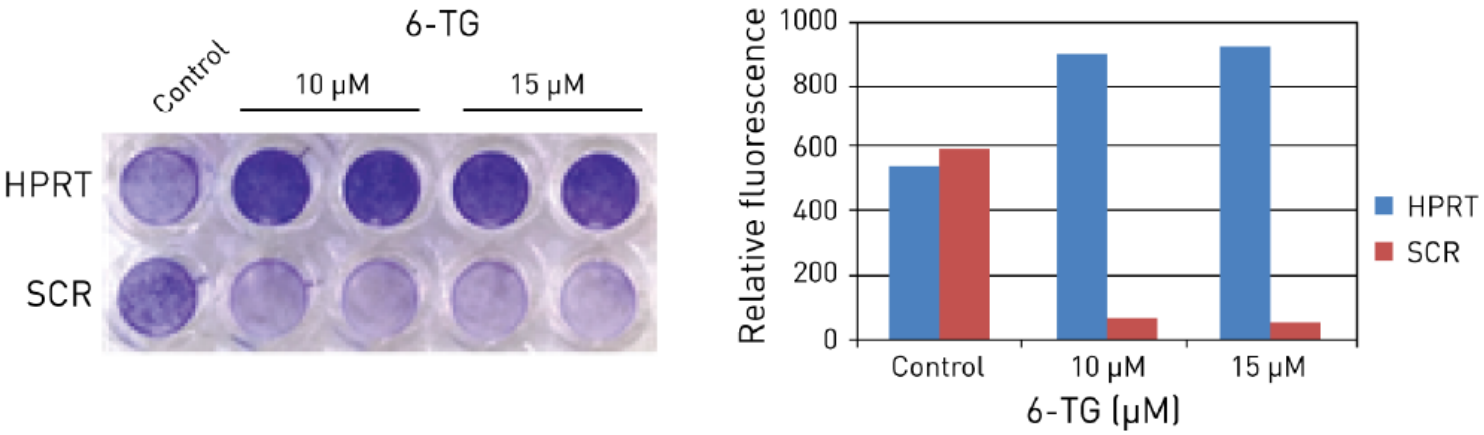


Fig. 3 Cas9 stable U2OS cells were transduced with LentiArray™ CRISPR Control Lentivirus particles expressing human HPRT1 (positive control) or non-targeting (negative control) gRNA sequences, then selected with puromycin. To verify functional HPRT1 knockout, the transductants were treated with or without 6-TG and analyzed by crystal violet staining or the PrestoBlue™ Cell Viability assay.

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.