shRNA transduction controls

OriGene offers two types of lenti shRNA transduction controls, GFP and RFP. The transduction controls can be used for monitoring transduction efficiency and also serves as non-target controls.

Products

Cat#	Product Name	Description
TR30021V	shRNA transduction control (GFP)	Control Lenti particles, shRNA scramble, expressing GFP and puro, 0.5 mL
TR30021V5		Control Lenti particles, shRNA scramble, expressing GFP and puro, 5x0.5 mL
TR30033V	shRNA transduction control (RFP)	Control Lenti particles, shRNA scramble, expressing RFP and blasticidin, 0.5 mL
TR30033V5		Control Lenti particles, shRNA scramble, expressing RFP and blasticidin, 5x0.5 mL

Features

- Prepackaged shRNA scramble particles, ready for transduction
- Puro or blasticidin selection marker driven by SV40
- GFP or RFP as a reporter

Applications

- Monitor transduction efficiency
- MOI optimization
- Non-target control

Specification

Titer $\geq 1x10^{7}$ TU/ml, by p24 ELISA

Storage and Stability

- Ship: dry ice
- Store: Aliquot and store at -80°C
- Stable for 6 months

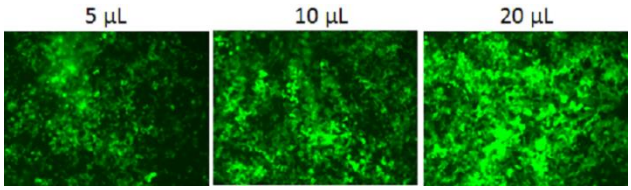

Fig. 1. HEK293 cells (96-well plate) were transduced with different volumes of TR30021V (GFP particles). Fluorescence pictures taken 72 hrs after.

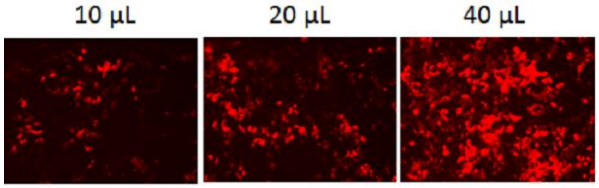

Fig. 2. HEK293 cells (96-well plate) were transduced with different volumes of TR30033V (RFP particles). Fluorescence pictures taken 72 hrs after.

For Research use Only, Not for use in diagnostic procedures.

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Protocol:

The following protocol is based on a 96-well plate. If your experiments require a different size of culture plates, just scale up or down the reagents accordingly based on the relative surface area of your plate.

Tissue Culture Vessel	Growth area, cm^2/well
96-well plate	0.35
24-well plate	2
12-well plate	4
6-well plate	9.5
35 mm plate	8
60 mm plate	20
100 mm plate	60

Day 1, seed cells

Seed $1x10^4$ HEK293T cells or your specific cells in each well of 96-well plate (Adjust the number of cells plated to 50% confluency upon transduction). Incubate 18–20 hours at 37°C in a humidified incubator in an atmosphere of 5% CO_2

Day 2, transduction

1. Calculate the amount of needed according to the desired multiplicity of infection (MOI).

2. Thaw the lentiviral shRNA particles on ice. Gently spin down before opening. Keep them on ice. Mix gently before use.

3. Remove medium from wells and add appropriate amount of Lentiviral shRNA particles, culture medium and polybrene (final concentration is 8 µg/mL) to the total volume of 100ul. Gently swirl the plate to mix.

4. Incubate 18–20 hours at 37°C in a humidified incubator in an atmosphere of 5% CO_2.

Overnight incubation may be avoided when toxicity of the lentiviral particles is a concern. (Cells may be incubated for as little as 4 hours before changing the medium containing lentiviral particles).

Note: When transducing a cell line for the first time, a range of volume or MOI should be tested. The transduction controls (cat# TR30021V or TR30033V) can be used for MOI optimization. MOIs of 1, 2, 5 and 10 or higher should be used to determine the optimal transduction efficiency and knockdown for each cell line.

Multiplicity of Infection (MOI):

Multiplicity of Infection (MOI) is the number of transducing lentiviral particles per cell.

To calculate:

(Total number of cells per well)x(Desired MOI) = Total transducing units needed (TU)

(Total TU needed)/(TU/mL) = Total mL of lentiviral particles to add to each well

Day 3, change media

Remove the medium containing lentiviral particles from wells and replace with 120 µLfresh pre-warmed complete culture medium to each well.

Day 5, Transduction efficiency or optimal MOI can be evaluated under the fluorescent microscope. If stable selection is needed, the cells can be splitted (1 to 10 dilution) and apply puromycin or blasticidin containing media.

Note: Perform a kill curve experiment for appropriate amount of puromycin or blasticidin used in each cell line.