Global UltraRapid Lentiviral Titer Kit

Cat # LV961A-1

(for Titering in Human and Mouse cells)

User Manual

Storage:
Store kit at -20°C

Version 6
8/31/2020

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Contents

Product Description

Pantropic VSV-G pseudotyped viral particles containing the lentivector expression construct can be used to efficiently deliver and stably express effector and reporter sequences in a wide range of mammalian target cells, but transduction efficiency can vary significantly depending on the transduction conditions and nature of target cells. Therefore, it is a standard procedure to determine the titer of the pseudovirus-containing supernatant in control HT1080 (human) or NIH-3T3 (mouse) cells before proceeding with transduction experiments in your target cells. After transduction of the lentiviral constructs into your target cells of interest, it is also necessary to confirm the transduction efficiency of your experiments. If a lentivector expression construct contains a GFP or RFP reporter, the percentage of infected cells can be easily determined as the percentage of GFP-or RFP-positive cells by fluorescence-activated cell sorting (FACS). However, the procedure requires a FACS machine, and it cannot be used if the vector does not contain a GFP or RFP marker. Additionally, the percentage of GFP- or RFP-positive cells does not always correlate with the number of infection-competent viral particles present in your viral preparations. This is because multiple viral particles can infect one single cell, especially when infection is conducted at high MOIs.

The Global UltraRapid Lentiviral Titer Kit rapidly determines the titer of pseudovirus particles by measuring the copy number of integrated lentiviral constructs in transduced target cells. By comparing the ratio of endogenous Ultra Conserved Region 1 (UCR1) DNA elements:lentivector-specific WPRE elements to a calibration curve generated with supplied standards, the Kit delivers highly quantitative measurement of the infectivity of the pseudoviral preparation.

Compatible with most WPRE-containing lentivector constructs—including pLVCT-H1, pLVTHM, pGIPz, HiPerform™ and all SBI lentivectors—the Kit contains all of the components needed to measure the number of endogenous UCR1 DNA elements and pseudo-lentiviral-specific WPRE elements. Even better, because the UCR1 primers are completely compatible with both human and mouse genomes, you can titer from either cell type.

The workflow is quick and simple:

1. Lyse infected cells (2 minutes at 95°C)
2. Add qPCR Mastermix from the Kit to the cleared lysate
3. Run a real-time PCR reaction (2 hours)
4. Analyze results

The manual does not include information on packaging lentivector constructs into pseudotyped viral particles or transducing your target cells of choice with the particles. This information is available in the user manual Lentivector Expression Systems: Guide to Packaging and Transduction of Target Cells, which is available on SBI's website (www.systembio.com). Before using the reagents and material supplied with this product, please read the entire user manual.

The kit contains calibration standards to measure titer, which can be used to calculate MOI. The calibration standards that are produced from WPRE-containing genomic DNA have been extensively calibrated with cells infected with a copGFP reporter construct at different MOIs. By calculating the amounts of WPRE and the internal UCR1 control amplified from your samples and the calibration standards, you can accurately determine the titer of the virus.

Some key terms used in the protocol:

MOI (multiplicity of infection): The ratio of infectious pseudoviral particles (IFU) to the number of cells being infected. IFU/ # cells = MOI.

IFU/ml (infectious units per ml): The relative concentration of infection-competent pseudoviral particles.

Transduction Efficiency: The average copy number of expression constructs per genome of target cell in the infected population.

List of Components

Table 1. Components of The Global UltraRapid Lentiviral Titer Kit

Components	Qty/Volume	Storage Temperature
25X Forward and Reverse WPRE Primer Mix	50 µL	-20˚C
25X Forward and Reverse UCR1 Primer Mix	50 µL	-20˚C
12.5X Calibration Standard 0 - Negative Control	20 µL	-20˚C
12.5X Calibration Standard 1	20 µL	-20˚C
12.5X Calibration Standard 2	20 µL	-20˚C

12.5X Calibration Standard 3	20 µL	-20˚C
12.5X Calibration Standard 4	20 µL	-20˚C
12.5X Calibration Standard 5	20 µL	-20˚C
2X SYBRTaq Mix	1.5 mL	-20˚C
Cell lysis buffer	5 ml	-20˚C

NOTE: The UltraRapid Lentiviral Titer Kit provides sufficient 2X SYBRTaq mix for 100 25-µl PCR reactions, enough for a maximum of 42 individual and singleplex titers

Handling the Reagents in the Titering kit

The reagents included in the titering kit are extremely sensitive to changes in temperature. Please note the following storage and handling conditions.

The SYBR Taq mixture should be aliquoted immediately upon receipt, and stored at -20ºC. The SYBR is light-sensitive, so please make sure to keep it in the dark for optimal performance.

The titering kit can be stored at -20 ºC for up to 1 year, however once thawed, the standards and primers should be kept at 4 ºC and should be used within one month.

The Standards, primers, and SYBR Taq are QCed and are lot specific for each lot of titering kit. Please do not substitute other brands of SYBR Taq, or mix and match standards and primers from other SBI kits and products.

Additional Required and Optional Equipment Not Included in Kit

Real time PCR System (Recommended: Applied Biosystems 7300 OR Real time PCR System, Cat# 4351101)

Protocol

Calculate a Standard Curve

Standards should be run in duplicate or triplicate so that an average of the ΔCt can be calculated. The standard curve should be run at the same time as the samples that are being titered. Please review all of the steps in the protocol before proceeding.

1. For each reaction, you will need 9.5 µL of PCR grade water, 12.5 µL of 2X SYBRTaq Mix, and 1 µL of 25X Primer Mix for either UCR1 or WPRE. Prepare two PCR master mixtures (one for UCR1 and the other for WPRE) enough for all reactions by doubling the volume of each ingredient with 2 plus the number of reactions. Combine the required volumes of PCR Grade Water, 2X SYBRTaq Mix, and the Primer Mix in order.
2. Mix contents by inverting the tubes a few times, and spin the tubes briefly in a microcentrifuge.
3. Aliquot 23 µL of the PCR Master Mix into each test tube or well (if you are using a 96-well plate).
4. Add 2 µL of each of the six control DNA calibration standards or the cell lysates from Step A into the test tubes/ wells from Step 3.
5. Seal the tubes or plate, and place them in the real time PCR system.
6. Commence thermal cycling using the following program: 50 °C for 2 min
    a. 95 °C for 10 min
    b. (95 °C for 15 sec; 60 °C for 1 min) for 40 cycles
    c. Add Dissociation step
7. When the program is complete, check the dissociation curve to make sure there is no significant contamination for WPRE amplification in the negative controls. Then export Ct to an Excel file and calculate the average Ct of UCR1 and WPRE for each standard and sample.
8. Calculate 2-ΔCt, where ΔCt = Average Ct of WPRE – Average Ct of UCR1 of the same standard or sample.
9. Use the Excel software to plot the MOIs* of the standards against the values of 2-ΔCt
10. Use the “add trendline” option of the software to draw the trendline of the standard curve. Set intercept at 0, check the boxes for Display Equation on chart” and “Display R- squared value on chart”.
11. Calculate MOI for each of your samples using the equation. For example, if the equation you obtain from your experiment is y = 1.192x, and 2-ΔCt of one of your samples is 5.1, the MOI of the sample should be 6.08 (i.e. 1.192 multiplied by 5.1).

*IMPORTANT: Please be aware that MOIs for each standard provided may vary from lot to lot. Refer to the tube of each standard for MOIs of the particular lot.

Titering Samples

IMPORTANT: The Global UltraRapid Lentiviral Titer Kit is compatible with both human and mouse cells.

1. Plate 50k cells/well in a 24 well-plate. Use 3 wells in duplicate, i.e. 6 wells.
2. Note: You need to determine the number of cells in a well of the plate upon infection. For HT1080 cells, the number of cells is around 75,000 per well if you plate 50,000 cells in each well of a 24-well plate 24 hours before infection.
3. Add 2 µl of concentrated virus in each of 2 wells.
4. Add 0.2 µl (dilute concentrate 1:10 then pipette 2 µl) into each of 2 wells.
5. Add 0.02 µl (diluted concentrate 1:100 the pipette 2 µl) into each of the 2 wells.
6. Transduce cells as indicated in the TransDux protocol.
7. Three days after infection, remove the medium, and carefully wash the cells in each well with 1 mL of PBS.

   IMPORTANT: If the cells have been transduced with unpurified pseudoviral stock (directly using viral supernatant from 293 cells), we recommend that after removing the medium containing the transfection reagent, you wash the transduced cells 3 times with fresh media and 1 time with PBS to remove lentiviral plasmid DNA impurities. These may be present in your cells due to residual transfer vector DNA from the 293 cell packaging step.
8. Remove, as much as possible, all of the PBS from the wells. Add 100 µL of lysis buffer to each well. At this point, you can either store the plate at -80 °C until ready to proceed or quick freeze the plate in dry ice and then thaw the plate at RT.
9. Detach the cells in each well by flushing with the lysis buffer and pipetting up and down the cell suspension a few times.
10. Transfer as much as possible of the lysed cells into a PCR tube.
11. Gently pipette up and down a few times to break down any visible cell clumps.
12. Heat the lysate at 95 °C for 2 minutes on a PCR machine.

13. Centrifuge the heated lysate at 14,000 RPM for 2 minutes and either put the tubes on ice or store at -20 °C until ready to be used.
14. Run the qPCR as described for the Standard Curve calculations.

The number of viral particles in your viral suspension (IFU/ml) can then be calculated with the following equation: (MOI of the sample) X (The number of cells in the well upon infection) X 1000 / (µl of viral suspension added to the well for infection).

Pilot Experiment on Target Cells: What is the Best MOI?

1. Plate target cells approximately 50K per well in 6 wells of a 24-well plate.
2. Transduce with high MOIs of 0, 1, 2, 5, 10, and 20.
3. The MOI with the highest reporter gene expression and healthiest cells is the appropriate amount to use.

UltaRapid qPCR titer setup

	Reaction vol	samples X	
	25.0	50.0	<- input # of samples here
Template	2.0		
MQ Water	9.5	475.0	
2X SYBR mix	12.5	625.0	
Primer Mix (25X)	1.0	50.0	UCR 1 or WPRE primers
to each well add	23.0		Then add 2 µl of either the standard or cell lysate to the wells as outlined below

Samples	1	2	3	4	5	6	7	8	9	10	11	12	
A	CS 0 (neg)	CS 1	CS 2	CS 3	CS 4	CS 5	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	UCR 1 Primer
B	CS 0 (neg)	CS 1	CS 2	CS 3	CS 4	CS 5	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12	UCR 1 Primer
C	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Sample 24	UCR 1 Primer
D	Sample 25	Sample 26	Sample 27	Sample 28	Sample 29	Sample 30	Sample 31	Sample 32	Sample 33	Sample 34	Sample 35	Sample 36	UCR 1 Primer
E	CS 0 (neg)	CS 1	CS 2	CS 3	CS 4	CS 5	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	WPRE Primer
F	CS 0 (neg)	CS 1	CS 2	CS 3	CS 4	CS 5	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12	WPRE Primer
G	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Sample 24	WPRE Primer
H	Sample 25	Sample 26	Sample 27	Sample 28	Sample 29	Sample 30	Sample 31	Sample 32	Sample 33	Sample 34	Sample 35	Sample 36	WPRE Primer

Figure 1. Example qPCR Setup

Example Data and Applications

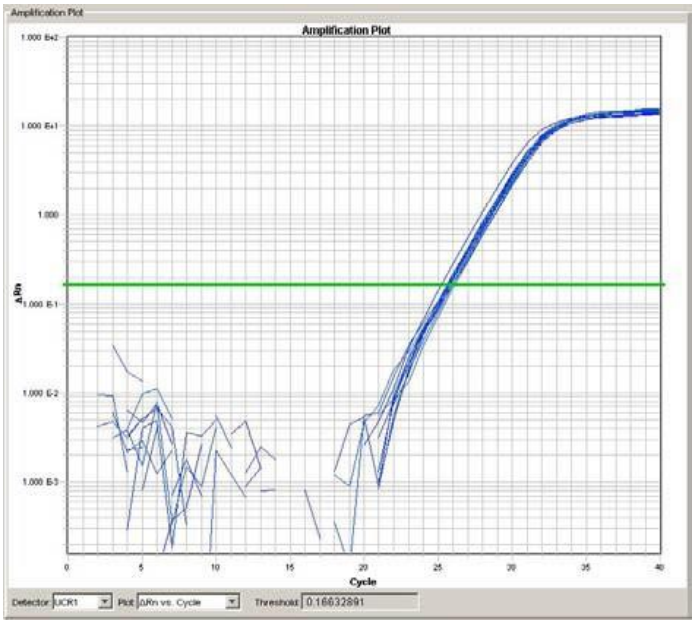

Figure 2. Example UCR Amplification Plot

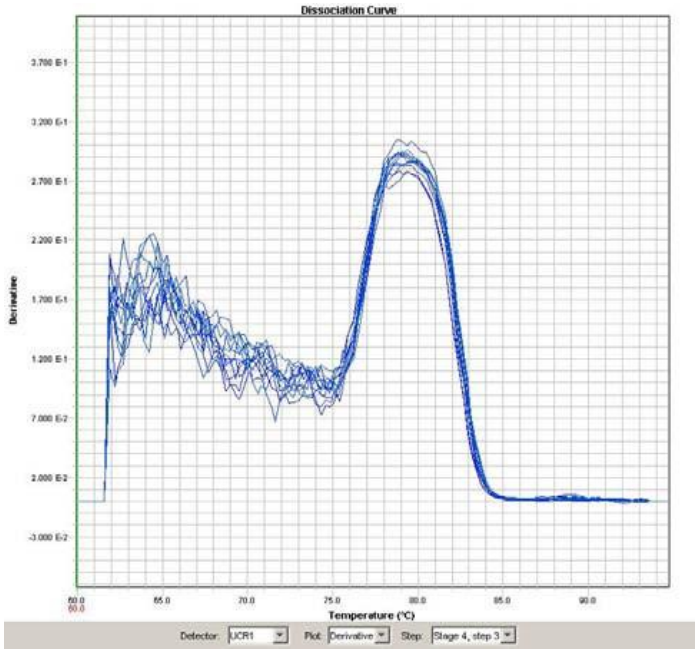

Figure 3. Example UCR Dissociation Curve

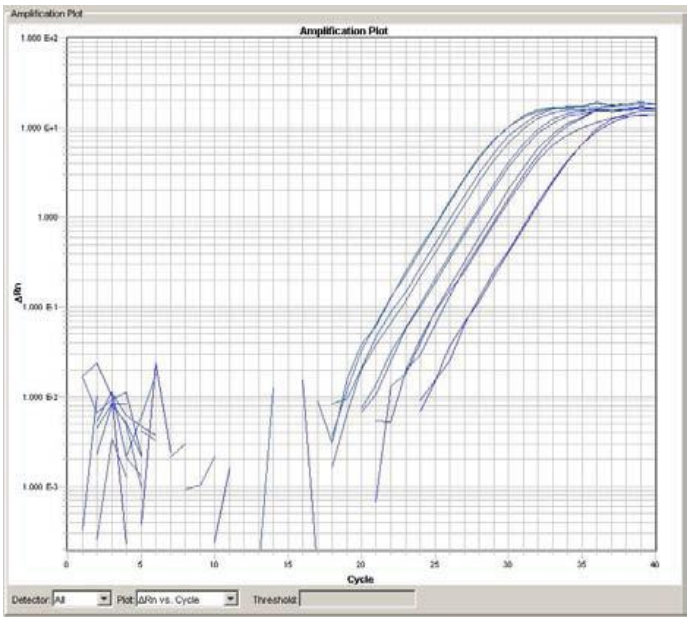

Figure 4. Example WPRE Amplification Plot

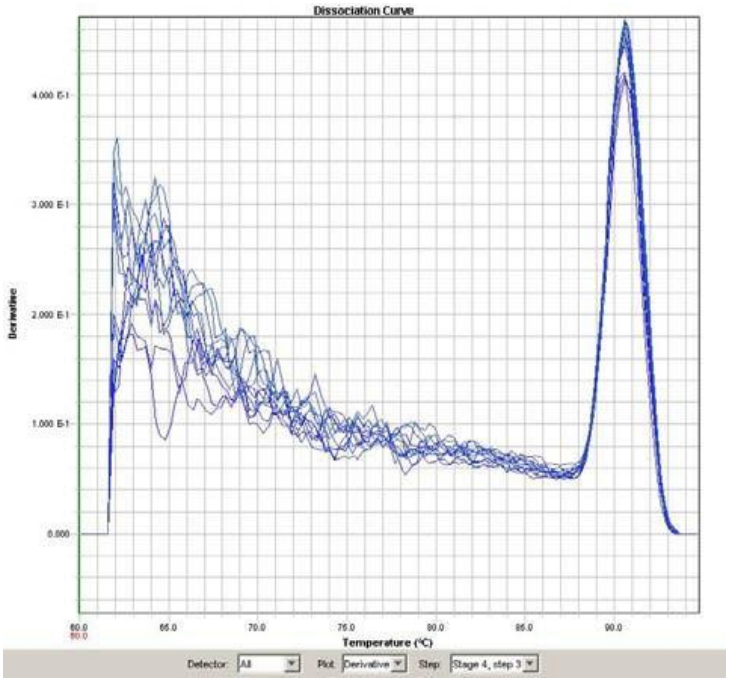

Figure 5. Example WPRE Dissociation Curve

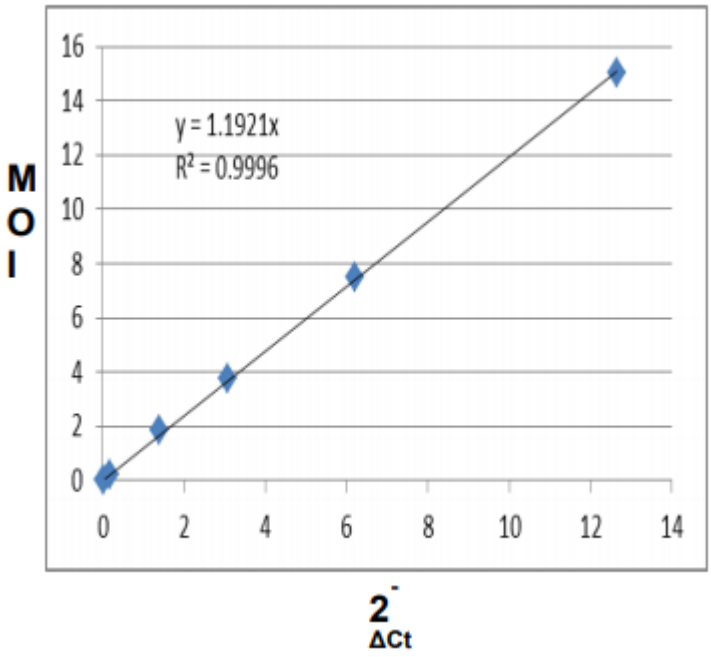

Figure 6. Example Standard Curve

Troubleshooting

A. No PCR product Amplified

- If no amplification from both calibration standard and sample DNA

  →Repeat PCR and make sure you have added all the components in the master mix.

- If no amplification from sample DNA only

  →The cells are not properly lysed. Make sure the cells are washed carefully with PBS and all residue amount of PBS is removed from the wells.

B. Dissociation Curves of Negative Controls are the Same as Those of Samples.

The negative controls are contaminated with a plasmid or sample containing WPRE in the lab. Make sure you apply all the cautions of PCR set-up to avoid contaminations. Especially, do not touch the inner lid of tubes, always use filtered tips, and avoid generate bubbles during pipetting.

C. MOI

- Using an MOI that is too high

  →This can result in an interferon response in the cells, causing the cells to look ailing.

- Using an MOI that is too low

  →This can result in an insufficient amount of construct to be expressed.

Technical Support

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