SHENTEK

Replication-Competent Lentivirus (RCL) Quantitation Kit

User Guide

Version: A/1
For Research Use Only
Product No.: 1403441
Reagents for 100 Reactions

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(IMPORTANT: Please read this document carefully before experiment.)

1. Product information

■ Product description

SHENTEK® Replication-Competent Lentivirus (RCL) Quantitation Kit is used for the quantitative detection of replicable lentiviral RCL in cell therapy products and gene therapy products produced with lentiviral vectors, such as virus-producing cell banks, end of production cells, viral vectors and CAR-T cells.

This kit is rapid, specific and reliable, and can work in coordination with the SHENTEK® Virus DNA & RNA Extraction Kit to quantitate the copy number of replicable lentivirus RCL in samples.

■ Kit contents and storage

WARNING: Please read the Material Safety Data Sheets (MSDSs) and follow the handling instructions. Wear appropriate protective eyewear, mask, clothing and gloves.

Table 1. Kit components and storage

Reagent	Part No.	Quantity	Storage
RCL Control	NNA044	lyophilized powder × 1 tube	-20℃
RCL Primer&Probe MIX	NNC077	300 μL × 1 tube	-20℃, protect from light
5×RT-qPCR Buffer	NNC078	600 μL × 1 tube	-20℃
RT-qPCR Enzyme MIX	NNC079	100 μL × 1 tube	-20℃, protect from light
RNase-Free H_2O	NND008	1.2 mL × 3 tubes	-20℃
ddH_2O	NND010	1 mL × 1 tube	-20℃

The kit components can be stored at appropriate conditions for up to 24 months. Please check the expiration date on the labels.

■ Applied instruments, including but not limited to the following

- SHENTEK-96S Real-Time PCR System
- 7500 Real-Time PCR System

- Lightcycler 480 II Real-Time PCR System
- CFX96 Real-Time PCR System
- qTOWER^3 G Real-Time PCR System

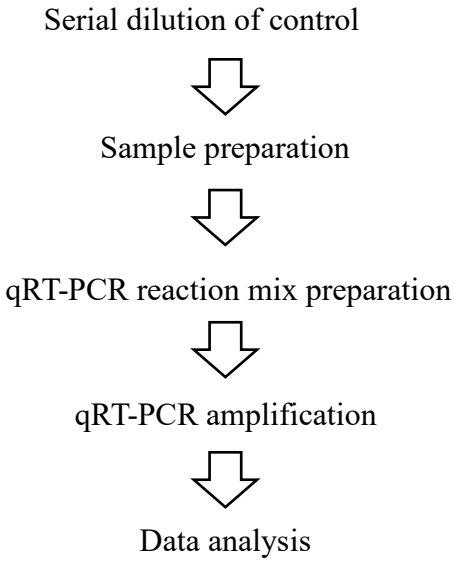
■ Required materials not included in the kit

- Nonstick, RNase-free, Low Retention Microfuge Tubes, 1.5 mL
- Nonstick, Low Retention Tips 1000 µL, 100 µL, 10 µL
- 96-well qPCR plates with sealing film or PCR 8-strip tubes with caps
- SHENTEK® Virus DNA & RNA Extraction Kit (Product No. 1506730)

■ Related equipment

- Benchtop microcentrifuge
- Vortex mixer
- Micropipettes 1000 µL, 100 µL and 10 µL
- Real-time PCR system
- Microplate shaker

■ Workflow



2. Methods

■ Experiment preparation

1. Wear appropriate protective eyewear, mask, clothing and gloves.
2. Irradiate the tabletop, micropipettes and tubes with UV for 30 minutes, and disinfect with 75% ethanol.
3. Thaw the kit completely at 2-8°C or melt on ice.

■ Dilution of RCL Control and preparation of standard curve

For the first use, spin RCL Control for 15 seconds in a microcentrifuge to collect lyophilized powder at the bottom of the tube. To dissolve the lyophilized powder, open the cap carefully and add 55 μL of ddH_2O to the bottom of the tube.

Note: Gently flick the RCL Control standard solution with finger several times, then spin for 3-5 seconds in a microcentrifuge. Repeat 3 times to fully dissolve the lyophilized powder in the solution.

Please check the concentration on the label of RCL Control tube prior to dilution.

1. Thaw RNase-Free H_2O completely at 2-8°C or melt on ice. Flick the RCL Control tube gently, and briefly centrifuge 3-5 seconds, and repeat 3 times to mix well.
2. Label eight nonstick 1.5 mL microfuge tubes: ST, ST0, ST1, ST2, ST3, ST4, ST5 and ST6, respectively.
3. Dilute the RCL Control to 6×10^8 copies/μL with RNase-Free H_2O in the ST tube. Vortex to mix well and quickly spin down the tube for 3-5 seconds in microcentrifuge, and mix thoroughly by repeating 3 times.
4. Add 90 μL RNase-Free H_2O to each tube of ST0, ST1, ST2, ST3, ST4 ST5 and ST6.
5. Perform the serial dilutions according to Table 2:

Table 2. Dilution for RCL Control

Serial dilution tube	Dilution	Conc. (copies/μL)
ST	Dilute the RCL Control with RNase-Free H_2O	6×10^8
ST0	10 μL ST + 90 μL RNase-Free H_2O	6×10^7
ST1	10 μL ST0 + 90 μL RNase-Free H_2O	6×10^6
ST2	10 μL ST1 + 90 μL RNase-Free H_2O	6×10^5
ST3	10 μL ST2 + 90 μL RNase-Free H_2O	6×10^4
ST4	10 μL ST3 + 90 μL RNase-Free H_2O	6×10^3
ST5	10 μL ST4 + 90 μL RNase-Free H_2O	6×10^2
ST6	10 μL ST5 + 90 μL RNase-Free H_2O	6×10^1

- *The remaining, unused RNase-Free H_2O need to be stored at 2-8℃. For long-term storage, please store at -20℃.*
- *At least five concentration of standard curve (ST2-ST6) should be included. To select appropriate sample dilutions, we recommend to perform method validation before sample testing.*

■ Sample preparation

➢ Negative Control Sample (NCS) Preparation

Add 100 μL of sample matrix solution (or RNase-Free H_2O) to a new 1.5 mL microcentrifuge tube, and label as NCS.

Note: The NCS should be processed along the same procedures as test sample preparation, from extraction to quantitative testing.

■ qRT-PCR MIX preparation

1. Determine the number of reaction wells based on your selected standard curve with the number of test samples and control samples. Generally, triplicates are tested for each sample.

   Number of reaction wells = (5 standard points on the standard curve + 1 NTC + 1 NCS + test samples)×3

2. Prepare qRT-PCR MIX according to the number of reaction wells in Table 3.

Table 3. qRT-PCR MIX Preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
5×RT-qPCR Buffer	6 μL	198 μL
RT-qPCR Enzyme MIX	1 μL	33 μL
RCL Primer&Probe MIX	3 μL	99 μL
Total volume	10 μL	330 μL

3. Mix thoroughly and place on ice, aliquot 10 μL/well into 96-well qPCR plates or PCR 8-strip tubes.

■ qRT-PCR Reaction MIX preparation

1. Prepare qRT-PCR Reaction MIX according to Table 4 and 96-well plates layout as shown in Table 5.

Table 4. qRT-PCR Reaction MIX Preparation

Tubes	Standard curve	NTC	NCS	Test sample
qRT-PCR MIX	10 μL	10 μL	10 μL	10 μL
Samples	20 μL ST2 - ST6	20 μL RNase-Free H_2O	20 μL purified NCS	20 μL purified test samples
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 5. Example of 96-well Plate layout

S1	S1	S1		NCS	NCS	NCS						A
S2	S2	S2		NTC	NTC	NTC						B
S3	S3	S3							ST6	ST6	ST6	C
									ST5	ST5	ST5	D
									ST4	ST4	ST4	E
									ST3	ST3	ST3	F
									ST2	ST2	ST2	G
												H
1	2	3	4	5	6	7	8	9	10	11	12	

- *This example represents the assay for a standard curve with 5 concentration gradients (ST2 to ST6), 1 NTC, 1 NCS, 3 test samples (S1 to S3), and 3 replicates*

for each sample.

- *In specific testing, the plate layout for sample loading can be adjusted based on the sample quantity. Please refer to the example shown in Table 5.*

2. Seal the 96-well plates with sealing film. Mix it well in microplate shaker, then spin down the reagents for 10 seconds and place it onto the qPCR instrument.

■ qRT-PCR program setting

NOTE: The following instructions apply only to the Applied Biosystems® 7500 instrument with SDS v1.4. If you use a different instrument or software, refer to the applicable instrument or software documentation.

1. Create a new document, then in the Assay drop-down list, select Standard Curve **(Absolute Quantitation)**.
2. In the Run Mode drop-down list, select **Standard 7500**, then click **Next.**
3. Click **New Detector:**
   a. Enter RCL in the Name field.
   b. Select **FAM** in the Reporter Dye drop-down list and select **none** in the Quencher Dye drop-down list, then click **OK**.
   c. Select a color for the detector.
   d. Select the detectors, then click **Add** to add the detectors to the document
4. Select **ROX** as the passive reference dye, then Click **Next**.
5. Select the applicable set of wells for the samples, then select RCL detector for each well.
6. Select Finish, and then set thermal-cycling conditions:
   a. Set the thermal cycling reaction volume to 30 μL.
   b. Set the temperature and time as following (Table 6).

Table 6. qRT-PCR running program

Step	Temp.	Time(mm:sec)	Cycles
Reverse transcription	50℃	15 :00	1
Activation	95℃	00 :30	1
Denature	95℃	00 :15	40
Anneal/extend	60℃ *	01 :00	

*Instrument will read the fluorescence signal during this step.

7. Save the document, then click **Start** to start the qRT-PCR run.

■ Results analysis

1. Select **Set up** tab, then set tasks for each sample type by clicking on the Task Column drop-down list:
   a. NTC: target DNA detector task = **NTC**
   b. NCS, test samples: target DNA detector task = **Unknown**
2. Set up the standard curve as shown in the following table (Table 7):

Table 7. Settings for Standard curve

Tube label	Task	Quantity (copies/μL)
ST2	Standard	6×10^5
ST3	Standard	6×10^4
ST4	Standard	6×10^3
ST5	Standard	6×10^2
ST6	Standard	6×10^1

3. Select the **Results** tab, then select Amplification Plot.
4. In the Data drop-down list, select **Delta Rn vs Cycle.**
5. In the Analysis Settings window, enter the following settings:
   a. Select **Manual Ct**.
   b. In the Threshold field, RCL enter 0.02.
   c. Select **Automatic Baseline**.
6. Click the button ▶ in the toolbar, then wait the plate analyzing.
7. Select the **Result** tab> >**Standard curve** tab, then verify the Slope, Intercept and

R^2 values.

8. Select the Report tab, then achieve the mean quantity and standard deviation for each sample.
9. Select **File > > Export > > Results**. In the Save as type drop-down list, select **Results Export Files**, then click **Save**.
10. In the Report panel of Results, the 'Mean Quantity' column shows the detection values of NTC, NCS, test sample, in copies/μL.
11. The Ct value of NTC should be no less than 35.00 cycles, meanwhile the Ct value of NCS should be larger than the mean Ct value of the lowest concentration in the standard curve.

Note: The parameter settings of the result analysis should be based on the specific model and the software version, and generally can also be automatically interpreted by the instrument.

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Support & Contact

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