SHENTEK

Reverse Transcriptase Assay Kit

User Guide

Version: A/0
For Research Use Only
Product No.: 1505700
Reagents for 50 Reactions

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

1. Product information

■ Product description

SHENTEK® Reverse Transcriptase Assay kit, in accordance with the reverse transcriptase activity test method in Chinese Pharmacopoeia, utilizes MS2 RNA as a template, followed by reverse transcription and fluorescent qPCR to detect the specific amplification signal.

This kit is suitable for the preparation of animal cell matrices during the production and verification of biological products. However, chicken embryonic fibroblasts (CEF) or other cells of avian origin, mice and other rodent-derived cell lines are often test positive for reverse transcriptase activity, because they contain retroviral gene sequences.In this regard, it is recommended to use other methods for testing in accordance with the requirements of laws and regulations.

■ 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
M-MLVRT Control	NNA024	6 μL × 1 tube	-20℃
ddH_2O	NND010	1 mL × 1 tube	
Buffer A	NND012	1.5 mL × 2 tubes	
Buffer B	NND013	750 μL × 1 tube	
MS2 RNA	NND011	15 μL × 1 tube	
Reverse transcription Buffer	NNB010	1 mL × 1 tube	-20℃, protect from light
MS2 Primer&Probe MIX	NNC036	150 μL × 1 tube	
2×qPCR SHENmix	NNC045	1 mL × 1 tube	
100×ROX	NND007	20 μL × 1 tube	

The kit components can be stored at appropriate conditions for up to 12 months.

Please check the expiration date on the labels.

■ Applied instruments, including but not limited to the following

- SHENTEK-96SReal-Time PCR System
- 7500 Real-Time PCR System
- CFX96 Real-Time PCR System
- LinGene 9600 Real-Time PCR System

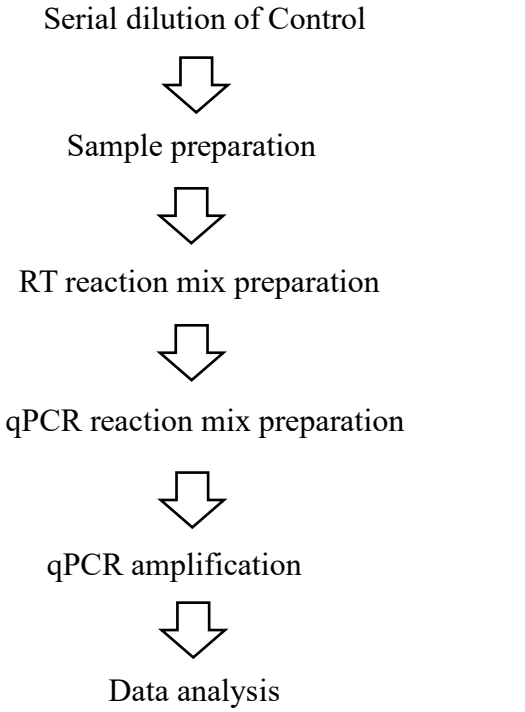
■ Required materials not included in the kit

- 10 mg/mL RNase A (User-supplied)
- Positive Control, contact us for order
- Nonstick, RNase-free & Low Retention Microfuge Tubes of 1.5 mL
- Nonstick, Low Retention Tips: 1000 µL, 100 µL and 10 µL
- 96-well qPCR plates with sealing film or PCR 8-strip tubes with caps

■ Related equipments

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

■ Workflow



2. Methods

■ Experiment preparation

1. Wear appropriate protective eyewear, mask, clothing and gloves.

2. Irradiate the tabletop, pipettes and tubes with UV for 30 minutes, and disinfect with 75% alcohol.

3. Thaw the kit completely at 2-8°C or melt on ice.

■ M-MLVRT Control serial dilutions for the standard curve

Please check the concentration on the label of the M-MLVRT Control (200U/μL) tube prior to dilution.

Prepare M-MLVRT Control solution with Buffer A following the serial dilution procedure below:

1. Thaw M-MLVRT Control and Buffer A completely at 2-8°C or melt on ice. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times.
2. Label nine nonstick 1.5 mL microfuge tubes: ST0, ST1, ST2, ST3, ST4, ST5,

ST6, ST7 and ST8.

3. Add 398 uL of Buffer A to the ST0 tube, and 45 uL of Buffer A to each of the remaining tubes.

4. Pipette 2 μL of M-MLVRT Control to the ST0 tube, insert the tips below the liquid level, and mix by blowing the pipette 10 times. Then mix again by vortex and quickly centrifuge for 3-5 seconds, and repeat vortex and centrifuge for 2 times to ensure sufficient mixing, and the prepared ST0 stock can be stable for one month when stored at -20℃.

5. Perform the serial dilutions according to Table 2.

Table 2. Dilution for M-MLVRT Control

Serial dilution tube	**Dilution**	**Conc.** (pU/μL)
ST0	2 μL M-MLVRT Control + 398 μL Buffer A	10^{12}
ST1	5 μL ST0 + 45 μL Buffer A	10^{11}
ST2	5 μL ST1 + 45 μL Buffer A	10^{10}
ST3	5 μL ST2 + 45 μL Buffer A	10^{9}
ST4	5 μL ST3 + 45 μL Buffer A	10^{8}
ST5	5 μL ST4 + 45 μL Buffer A	10^{7}
ST6	5 μL ST5 + 45 μL Buffer A	10^{6}
ST7	5 μL ST6 + 45 μL Buffer A	10^{5}
ST8	5 μL ST7 + 45 μL Buffer A	10^{4}

- *At least five concentration of standard curve should be included. Normally, standard concentration of ST3 - ST8 are recommende in this assay.*
- *Users should carry out the detection system sensitivity test for the first time to use this product. Take ST8 as test sample, peform reverse transcription repeats of 10 tubes, followed by single qPCR detection for each tube, in total of 10 wells.*

■ Sample preparation

➢ Test sample preparation

Take 200 μL of the test sample, centrifuge for 5min, 5000 rpm/min, take 20 μL of

the supernatant, add 20 μL of Buffer B and mix well, then placed in an ice bath for 15 min before use, and store at -70℃ in case of delayed use.

- **Reverse transcription test sample preparation**

  Take 5 μL of the processed sample, add 45 μL of Buffer A and mix by blowing the pipette 10 times, vortex and briefly spin for 3-5 seconds, and repeat vortex and spin for 2 times to ensure adequate mixing. Samples can be sequentially diluted to the appropriate concentration.

■ Positive and negative control preparation

- Positive control (PC): Contact us to order. PC should be prepared in same way as test samples. Aliquot PC stock in single doses and store at -65℃.
- Negative control: Buffer A, should be prepared in the same way as test samples as well.

■ RT Reaction MIX preparation

1. Determine the number of reaction wells based on your selected standard curve, with the number of test samples and control samples.

   Number of reaction tubes = Standard curve of 6 concentration gradients + 1 PCS + 1 NCS + N test samples (An additional 10 tubes of sensitivity test samples should be included for the first use.)

2. Prepare RT-MIX according to the number of reaction tubes, as shown in table 3.

Table 3. RT-MIX preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
Reverse transcription Buffer	19.7 μL	650.1μL
MS2 RNA	0.3 μL	9.9 μL
Total volume	20 μL	660 μL

3. Incubated the RT-MIX at 70℃ for 10 min,and place on ice immediately for at least 5 min.Then vortex and spin for 3-5 seconds, and repeat for 2 times, Then aliquot 20 μL/well into 96-well qPCR plate, PCR 8-strip tubes or 1.5 mL low retention microfuge tubes.

4. Prepare RT Reaction MIX according to Table 4. Add corresponding samples to RT-MIX, mix 10 times by blowing the pipette 10 times, cap or seal the tube tightly, vortex and breif spin for 3-5s. The total volume of each reaction is 25 uL.

Table 4. RT Reaction MIX preparation

Tubes	Standard curve	PCS	NCS	Test sample	Sensitivity sample
RT-MIX	20 μL	20 μL	20 μL	20 μL	20 μL
Samples	5 μL ST3 - ST8	5 μL Positive control	5 μL Buffer A	5 μL Reverse transcription test sample	5 μL Sensitivity samples

5. Incubate the reaction tube at 37℃ for 4h until the reverse transcription product is obtained. Vortex to mix and rapidly centrifuge for 3-5s, and immediately proceed to the qPCR step or store overnight at -20℃ to continue the next day.

■ qPCR Reaction MIX preparation

1. Calculate the number of qPCR reaction wells required based on the number of RT reaction tubes, generally 3 replicate wells should be included for each RT reaction tube:

qPCR reaction wells = RT reaction tubes × 3

2. Prepare qPCR Reaction MIX according to the number of reaction wells

Table 5. qPCR Reaction MIX preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
2×qPCR SHENmix	15 μL	495 μL
MS2 Primer&Probe MIX	3 μL	99 μL
RNase A (10mg/mL, User-supplied)	1 μL	33 μL
100×ROX	0-0.3 μL	0-9.9 μL
ddH_2O	to 25 μL	to 825 μL

- *qPCR Reaction MIX contains RNaseA, and prepare and spiking should be separated from the reverse transcription operation area.*

- *The dosage of ROX could be determined according to the qPCR instrument manufacturer's recommendations, e.g., the dosage of model 7500 Real-Time PCR system is 0.1 μL, and the dosage of model CFX Real-Time PCR system is 0 μL.*
- *The remaining unused 100×ROX need to be stored at 2-8℃, and protect from light.*

3. Add 25 μL/well of qPCR Reaction MIX into a 96-well plate as shown in Table 6, and pipette 5 μL of reverse transcription product to the corresponding wells. The total volume of each reaction is 30 μL/well.

Table 6. Example of 96-well plate layout

PCS				S1	S1	S1			ST3	ST3	ST3	A
PCS				S2	S2	S2			ST4	ST4	ST4	B
									ST5	ST5	ST5	C
									ST6	ST6	ST6	D
								ST8-RT7	ST7	ST7	ST7	E
								ST8-RT8	ST8	ST8	ST8	F
NCS								ST8-RT9	ST8-RT1	ST8-RT2	ST8-RT3	G
NCS								ST8-RT10	ST8-RT4	ST8-RT5	ST8-RT6	H
1	2	3	4	5	6	7	8	9	10	11	12	

- *This example represents assays, including selected standard curve points of* Reverse transcription *(ST3～ST8), 10 sensitivity ST8 (from reverse transcription reaction RT1-RT10), 1 NCS, 1 PCS and 2 test sample(S1-S2), with 3 replicates for each sample except for sensitivity test samples.*
- *The plate layout for sample loading can be adjusted based on the sample quantity.*

4. Seal the 96-well plate with sealing film. Mix well in microplate shaker, then spin down the reagents for 20 seconds in microcentrifuge and place it on the qPCR instrument.

■ qPCR program setting

NOTE: The following instructions apply only to the ABI7500 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 RT 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, then click **Create Another**.

4. Select **ROX** as the passive reference dye, then Click **Next**.

5. Select the applicable set of wells for the samples, then select the corresponding 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 follow in Table 7:

Table 7. qPCR running temperature and time

Step	Temp.	Time(mm:sec)	Cycles
RNase digestion	37℃	07:00	1
Activation	95℃	05:00	1
Denature	95℃	00:20	50
Anneal	57℃*	01:00	
Extend	72℃	00:10	
Extend	72℃	02:00	1

* *Instrument will read the fluorescence signal during this step.*

7. Save the document, then click **Start** to start the qPCR 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= **Unknown**
2. Set up the standard curve as shown in table 8:

Table 8. Settings for Standard curve

Tube label	Task	Quantity (pU/μL)
ST3	Standard	10^9
ST4	Standard	10^8
ST5	Standard	10^7
ST6	Standard	10^6
ST7	Standard	10^5
ST8	Standard	10^4

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, 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 NCS, PCS, test sample, in pU/μL.

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.

■ Results determination

1. Criteria for method validation for detection sensitivity

All 10 test samples of 10^4 pU/μL (ST8) should be detected.

2. Experimental validity

The standard curve R^2 is not less than 0.96, the PCS Ct ≤ 28, and the sensitivity sample Ct ≤ 38, then the test is considered to be qualified.

3. Determination of the test sample results

(1) If there is no Ct value result for the test sample, or the Ct value is ≥ 40 with no obvious amplification curve, the reverse transcriptase activity of test sample can be determined as negative.

(2) If the test sample Ct value < 40 and shows a clear amplification curve, the reverse transcriptase activity can be calculated according to the following formula:

Reverse transcriptase activity in the sample (pU/mL) = A × D × 1000

(A is the mean value of the repeats, pU/μL; D is the sample dilution factors, D=20.)

(3) If the Ct value of test sample is located between 38 - 40, we recommend to repeat the test one more time. If the Ct value of the repeated measurement is <40 and there is a clear amplification curve, then determine as positive.

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Support & Contact

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