SHENTEK

Residual HPV18 E6/E7 DNA Size Analysis Kit

User Guide

Version: A/0
For Research Use Only
Product No.: 1103182
Reagents for 4×100 Reactions

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

1. Product information

■ Product description

SHENTEK® Residual HPV18 E6/E7 DNA Size Analysis Kit is used to quantitate residual HPV18 E6/E7 DNA of different fragment sizes of HeLa cell origin at various stages of biopharmaceutical products, from in-process samples to final products.

This kit utilizes real-time PCR technique to perform rapid and specific quantitation of residual HPV18 E6/E7 DNA fragments (FAM) in samples. It is designed to amplify four different fragments (E6:100bp and 288bp; E7:110bp and 240bp) for the accurate determination of their size distribution. For extraction information, please refer to the SHENTEK® Residual Host Cell DNA Sample Preparation Kit User Guide (Product No. 1104191).

■ 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
I	IPC MIX	NNC066	150 µL×1 tube	-20℃, protect from light
	E6-100 primer&probe MIX	NNC055	300 µL×1 tube	
	E6-288 primer&probe MIX	NNC056	300 µL×1 tube	
	qPCR Reaction Buffer	NNB001	850 µL×5 tubes	
	DNA Dilution Buffer (DDB)	NND001	1.5 mL×2 tubes	-20℃
	HPV E6/E7 DNA Control	NNA030	50 µL×1 tube	
II	E7-110 primer&probe MIX	NNC057	300 µL×1 tube	-20℃, protect from light
	E7-240 primer&probe MIX	NNC058	300 µL×1 tube	
	qPCR Reaction Buffer	NNB001	850 µL×4 tubes	
	DNA Dilution Buffer (DDB)	NND001	1.5 mL×2 tubes	-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
- ABI 7500 Real-Time PCR System
- CFX96 Real-Time PCR System
- LineGene 9600plus Real-Time PCR System

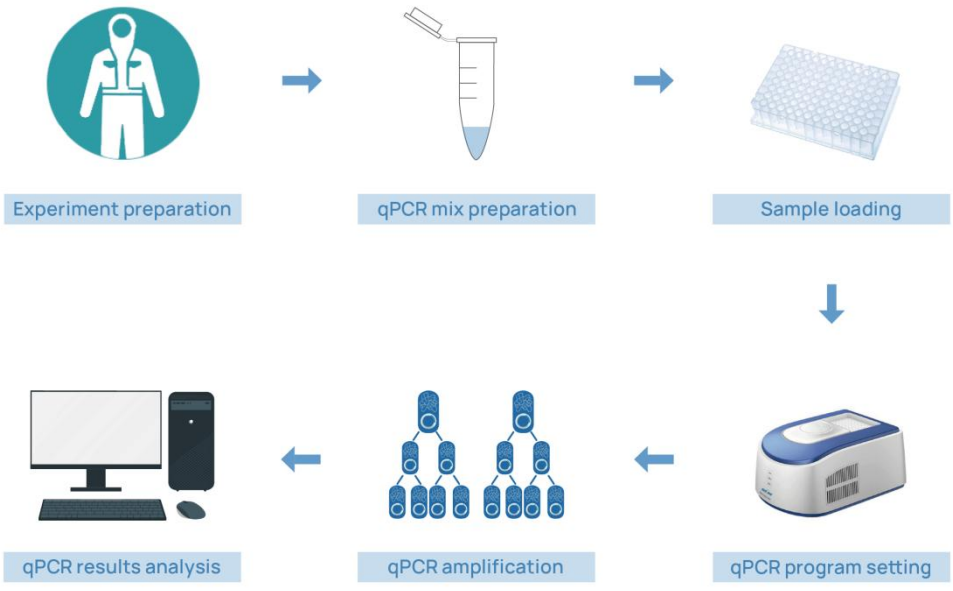
■ Required materials not included in the kit

- Low retention, RNase/DNase-free, sterile microcentrifuge tubes
- PCR 8-well strip tubes with caps or 96-well plate with seals
- Low retention filter tips: 1000 µL, 100 µL and 10 µL
- ddH_2O

■ Related equipments

- Real-Time PCR System
- Benchtop microcentrifuge
- Vortex mixer
- Pipettes: 1000 µL, 100 µL and 10 µL
- Microplate and microtube 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% ethanol.
3. Thaw the kit completely at 2-8°C or melt on ice, vortex and spin briefly.

■ DNA Control serial dilutions for the standard curve

Note: The kit contains four HPV E6/E7 primer & probe mixes for different fragment lengths. Please set up four separate standard curves corresponding to each fragment length.

Please check the concentration labeled on the tube containing the HPV E6/E7 DNA Control prior to dilution.

Prepare four sets of HPV18 E6/E7 DNA Control solution with DNA Dilution Buffer (DDB) following the serial dilution procedure below:

1. Thaw HPV18 E6/E7 DNA Control and DDB 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 seven1.5 mL microcentrifuge tubes: ST0, ST1, ST2, ST3, ST4, ST5 and ST6.
3. Dilute the DNA Control to 1×10^8 copies/μL with DDB in ST0 tube. Calculate the volume of DDB to prepare the ST0:

$$\frac{\text{DNA Control conc. (A)} \times \text{Volume of DNA Control(B)}}{1 \times 10^8 \text{ copies/μL}} - \text{Volume of DNA Control(B)}$$

For example:

The concentration on the label of the DNA Control is 7.0×10^9 copies/μL (A), pipette 10 μL (B) of the DNA Control to the ST0 tube. Add the below volume to reach 1×10^8 copies/μL.

$$\frac{7.0 \times 10^9 \text{copies/μL} \times 10\ \text{uL}}{1 \times 10^8\ \text{copies/μL}} - 10\ \text{uL} = 690\ \text{uL}$$

4. Vortex to mix well and quickly spin down the ST0 tube for 3-5 seconds in microcentrifuge, and repeat 3 times to mix it thoroughly.

5. Add 180 μL DDB to each tube of ST1, ST2, ST3, ST4, ST5 and ST6.

6. Perform the serial dilutions according to Table 2:

Table 2. Dilution for HPV E6/E7 DNA Control

Serial dilution tube	Dilution	Conc. (copies/μL)
ST0	Dilute the DNA Control with DDB	1×10^8
ST1	20 μL ST0 + 180 μL DDB	1×10^7
ST2	20 μL ST1 + 180 μL DDB	1×10^6
ST3	20 μL ST2 + 180 μL DDB	1×10^5
ST4	20 μL ST3 + 180 μL DDB	1×10^4
ST5	20 μL ST4 + 180 μL DDB	1×10^3
ST6	20 μL ST5 + 180 μL DDB	1×10^2

- *The remaining unused DDB need to be stored at 2-8℃. If the solution is cloudy or contains precipitates, heat at 37℃ until it clear.*
- *At least five concentrations of the standard curve should be included. To select appropriate sample dilutions, we recommend performing method validation before sample testing.*

■ Sample preparation

➢ Negative Control Sample (NCS) Preparation

Add 100 μL of DDB to a new 1.5 mL microcentrifuge tube, and label as NCS.

NCS should be processed in the same procedures as test sample preparation before testing.

■ qPCR MIX preparation

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

Number of reaction wells = (6 standard points on the standard curve + 1 NTC + 1

NCS + test samples)×3

2. Prepare qPCR MIX according to the number of reaction wells in Table 3-6.

Table 3.E6-100 qPCR MIX preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	17 μL	561 μL
E6-100 primer&probe MIX	3 μL	99 μL
Total volume	20 μL	660 μL

Table 4.E6-288 qPCR MIX preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	17 μL	561 μL
E6-288 primer&probe MIX	3 μL	99 μL
Total volume	20 μL	660 μL

Table 5. E7-110 MIX qPCR preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	17 μL	561 μL
E7-110 primer&probe MIX	3 μL	99 μL
Total volume	20 μL	660 μL

Table 6. E7-240 MIX qPCR preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	17 μL	561 μL
E7-240 primer&probe MIX	3 μL	99 μL
Total volume	20 μL	660 μL

For simultaneous detection of the four fragments, please prepare at least 120 μL template DNA for four assays.

3. Mix thoroughly and place on ice, aliquot 20 μL/well into 96-well qPCR plate or PCR 8-strip tubes.

■ qPCR Reaction MIX preparation

1. Prepare qPCR Reaction MIX according to table 7-10.

Table 7. E6-100 qPCR Reaction MIX preparation

Tubes	Standard curve	NTC	NCS	Test sample
E6-100 qPCR MIX	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST6	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 8. E6-288 qPCR Reaction MIX preparation

Tubes	Standard curve	NTC	NCS	Test sample
E6-288 qPCR MIX	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST6	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 9. E7-110 qPCR Reaction MIX preparation

Tubes	Standard curve	NTC	NCS	Test sample
E7-110 qPCR MIX	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST6	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 10. E7-240 qPCR Reaction MIX Preparation

Tubes	Standard curve	NTC	NCS	Test sample
E7-240 qPCR MIX	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST6	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

■ IPC Reaction MIX preparation

1. IPC for NTC and Test samples are required for each test. Prepare IPC qPCR MIX and IPC Reaction MIX according to Table 11 and 12.

Table 11. IPC qPCR MIX Preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
qPCR Reaction Buffer	15.9 μL	524.7 μL
IPC MIX	1.3 μL	42.9 μL
ddH_2O	2.8 μL	92.4 μL
Total volume	20 μL	660 μL

Table 12. IPC Reaction MIX Preparation

Tubes	IPC for Test sample	IPC for NTC
IPC qPCR MIX	20 μL	20 μL
Samples	10 μL purified test sample	10 μL DDB
Total Volume	30 μL	30 μL

2. 96-well plate layout template of E6-100 and E6-288 are shown in Table 13.

Table 13. Example of 96-well plate layout

E6-100						E6-288						
NTC	NTC	NTC	S1	S1	S1	S1	S1	S1	NTC	NTC	NTC	A
NCS	NCS	NCS	S2	S2	S2	S2	S2	S2	NCS	NCS	NCS	B
ST6	ST6	ST6							ST6	ST6	ST6	C
ST5	ST5	ST5							ST5	ST5	ST5	D
ST4	ST4	ST4							ST4	ST4	ST4	E
ST3	ST3	ST3	IPC-S1	IPC-S1	IPC-S1				ST3	ST3	ST3	F
ST2	ST2	ST2	IPC-S2	IPC-S2	IPC-S2				ST2	ST2	ST2	G
ST1	ST1	ST1	IPC-NTC	IPC-NTC	IPC-NTC				ST1	ST1	ST1	H
1	2	3	4	5	6	7	8	9	10	11	12	

- *This example represents E6-100 and E6-288 assays, including selected standard curve points of HPV18 E6/E7 DNA Control (ST1-ST6),1 NTC,1 NCS, 2 test*

samples(S1,S2), 1 IPC-NTC, 2 IPC-samples (IPC-S1, IPC-S2) and 3 replicates for each sample.

- *The plate layout for sample loading can be adjusted based on the sample quantity.*

3. Seal the 96-well plate with sealing film. Mix well in microplate shaker, then spin down the reagents for 10 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. Click **New Detector**, then enter E6-100 in the Name field, select **FAM** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.

3. Create new detector for E6-288, E7-110 and E7-240, separately as step2.

4. Click **New Detector**, then enter **IPC** in the Name field. Select **VIC** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.

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

6. Select the applicable set of wells for the samples, then select the corresponding detector for each well.

7. 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 in Table 14:

Table 14. qPCR running temperature and time

Step	Temp.	Time(mm:sec)	Cycles
Activation	95℃	10:00	1
Denaturation	95℃	00:15	40
Annealing/Extension	60℃*	01:00	

*Instrument will read the fluorescence signal during this step.

8. 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 the following table 15:

Table 15. Settings for Standard curve

Tube label	Task	Quantity (copies/μL)
ST1	Standard	1×10^7
ST2	Standard	1×10^6
ST3	Standard	1×10^5
ST4	Standard	1×10^4
ST5	Standard	1×10^3
ST6	Standard	1×10^2

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**.

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.

10. Analyze the Ct value of IPC. Normally, the mean Ct-IPC value of the sample should be within ±1.0 of the NCS Ct-IPC value. If the mean Ct-IPC value of the sample is significantly higher than the Ct-IPC value of the NCS, this indicates that the sample may be inhibitory to the assay. We recommend to test the ERC samples at the same assay, and take the sample recovery rate as the criterion.

Effective date: 28 May 2025

Support & Contact

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