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Residual HPV18 E6/E7 DNA Size Analysis Kit User Guide

Version: A/2

Product No.: SK030307S-P

Reagents for 4×100 Reactions

For Research Use Only. Not for diagnostic or therapeutic use.

SHENTEK® Residual HPV18 E6/E7 DNA Size Analysis Kit

(PCR-Fluorescence Probe Method)

Product No.: SK030307S-P

Reagents for 4×100 Reactions

WARNING! Read this user guide thoroughly before starting the experiment. Pay close attention to the precautions and frequently asked questions.

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, mask, clothing and gloves.

Product information

Product description

The **SHENTEK® Residual HPV18 E6/E7 DNA Size Analysis Kit** serves to the quantitative detection of the residual HPV18 E6/E7 DNA of different fragment sizes of HeLa cell origin derived from various biologic and pharmaceutical processes such as in-process samples, bulk harvest, and final product.
This kit utilizes a fluorescent probe-based qPCR principle to quantitatively detect specific genomic regions of host cell, providing rapid detection with high specificity and reliable performance, limitation of detection reaches the femtogram (fg) level for HPV18 E6/E7 residual DNA in various sample types.It is designed to amplify four different fragments (E6:100bp and 288bp; E7:110bp and 240bp) for the accurate determination of their size distribution.

Compatible Kit:

- SHENTEK® Residual Host Cell DNA Sample Preparation Kit (Fast) (Product No.: 1104201)
  Enable efficient recovery of residual DNA from a broad range of products across different manufacturing stages.

Kit components and storage

Reagent		Part No.	Volume	Storage
I	HPV E6/E7 DNA Control	NNA030	1 × 50 µL	-20°C*
	DNA Dilution Buffer (DDB)	NND001	2 × 1.5 mL	-20°C
	qPCR Reaction Buffer	NNB001	5 × 850 µL	-20°C, protect from light
	E6-100 primer&probe MIX	NNC055	1 × 300 µL	-20°C, protect from light
	E6-288 primer&probe MIX	NNC056	1 × 300 µL	-20°C, protect from light
	IPC MIX	NNC066	1 × 150 µL	-20°C, protect from light
II	DNA Dilution Buffer (DDB)	NND001	2 × 1.5 mL	-20°C
	qPCR Reaction Buffer	NNB001	4 × 850 µL	-20°C, protect from light
	E7-110 primer&probe MIX	NNC057	1 × 300 µL	-20°C, protect from light
	E7-240 primer&probe MIX	NNC058	1 × 300 µL	-20°C, protect from light

*-30°C to -10°C is acceptable per USP <659>. Up to 24 months storage under proper conditions. Check the label for expiry date.

Materials required

Compatible Instruments (including but not limited to)	SHENTEK-96H Real-time PCR System (Product No.: 161086002)
	ABI 7500 Real-Time PCR System
	CFX96 Real-Time PCR System
	LineGene 9600 Plus Real-Time PCR System
Equipments	Benchtop microcentrifuge
	Vortex mixer
	Pipettes
Consumables	Low retention, DNase-free, Sterile microcentrifuge tubes, 1.5 mL
	8-tube PCR strips or 96-well PCR plates
	Low retention, Sterile filter tips: 1000 µL, 100 µL and 10 µL
	ddH_2O

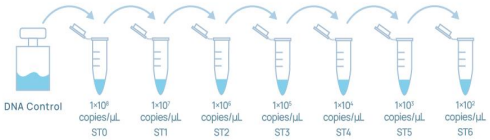
Quick Process Flow Char

01 Dilution of DNA Control materials and preparation of standard curves

- DNA Control
- DNA Dilution
- Primer & Probe MIX
- qPCR Reaction Buffer
- IPC MIX

Vortex

Centrifuge briefly for 3~5 seconds



Four separate standard curves.
Preparation of quantitative participation sample recovery quality control ERC.

02 Preparation of qPCR & IPC premix

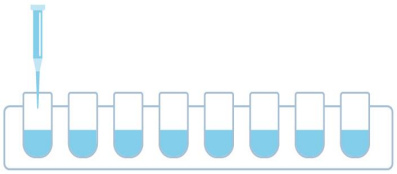
Four separant qPCR MIX preparation (example for a single well)

- Add 17 μL qPCR Reaction Buffer
- Add 3 μL Primer & Probe MIX

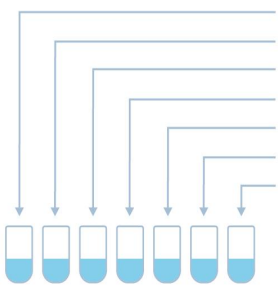
IPC MIX preparation (example for a single well)

- Add 15.9 μL qPCR Reaction Buffer
- Add 1.3 μL IPC MIX
- Add 2.8 μL ddH_2O

Add 20 μL qPCR & IPC MIX to each well



03 Sample addition



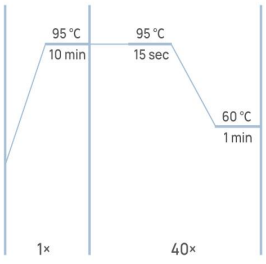
NTC (add 10 μL DNA dilution)
NCS (add 10 μL NCS purification solution)
Sample (add 10 μL sample purification solution)
ERC (add 10 μL ERC purification solution)
ST (add 10 μL standard solution)
IPC-NCS (add 10 μL IPC-NCS purification solution)
IPC-Sample (add 10 μL IPC-sample purification solution)

Tightly cover the tube cap or seal with a sealing film

Centrifuge for 10 seconds

Run samples in triplicates

04 qPCR Program Setup and Operation



Analysis procedure

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.
4. If the DNA Dilution Buffer (DDB) solution is cloudy or contains precipitates, heat at 37°C until it clears.

DNA Control serial dilutions for the standard curve

- At least five concentration values should be included when creating a standard curve. To select appropriate sample dilutions, we recommend performing method validation before sample testing.

1. Thaw DNA Control and DDB completely. Vortex and quickly spin down, repeat 3 times.
2. Prepare and label 1.5 mL microcentrifuge tubes as ST0, ST1, ST2, ST3, ST4, ST5 and ST6, respectively.
3. Dilute DNA Control with DNA Dilution Buffer in the tube ST0 to 1×10^8 copies/μL. Calculate the volume of DDB to prepare the ST0:

$$\frac{\text{DNA Control conc.(A)} \times \text{Volume of DNA Control (B)}}{1\times10^8 \text{ copies/ μL}} - \text{Volume of DNA Control (B)}$$

Example Calculation:

For 10 μL DNA Control (7.0×10^9 copies/μL), 690 μL of DDB will be added.

$$\frac{7.0\times10^9 \text{ copies/μo} \times 10\ \mu\text{L}}{1\times10^8 \text{ copies/ μL}} - 10\ \mu\text{L} = 690\ \mu\text{L}$$

4. Add 180 μL of DDB to tubes ST1, ST2, ST3, ST4, ST5 and ST6, respectively. After each transfer, vortex thoroughly, then centrifuge briefly.
5. Perform serial dilution according to the table below.

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

ERC (Extraction Recovery Control)

Aliquot 100 μL of the test sample to a new 1.5 mL microcentrifuge tube and mix thoroughly with 10 μL of ST3 solution. Label the tube with “ERC”. The ERC concentration may be adjusted according to the actual testing requirement.

NCS (Negative Control Sample)

Add 100 μL of DDB to a new 1.5 mL microcentrifuge tube. Label the tube with “NCS”.

Note: Both ERC and NCS should share the same preparation procedures with the test samples.

qPCR Reaction MIX & IPC Reaction MIX preparation

1. Based on the number of standard curve points, samples, and control samples, calculate the total number of reaction wells required for the assay. Triplicate recommended for each sample.

Number of reaction wells = (6 standard curve points + 1 NTC + 1 NCS + test samples) × 3

2. Thaw all components completely and thoroughly mix the reagents and briefly centrifuge. Prepare the qPCR MIX &

IPC MIX according to the reagents and volumes shown in the table below. Ensure thorough mixing and briefly centrifuge after preparation.

qPCR Reaction MIX preparation

Component	Sample volume (1 well)	Sample volume (30 wells) *
qPCR Reaction Buffer	17 μL	561 μL
E6-100 Primer&Probe MIX	3 μL	99 μL
Total volume	20 μL	660 μL
Component	**Sample volume (1 well)**	**Sample volume (30 wells) ***
qPCR Reaction Buffer	17 μL	561 μL
E6-288 Primer&Probe MIX	3 μL	99 μL
Total volume	20 μL	660 μL
Component	**Sample volume (1 well)**	**Sample volume (30 wells) ***
qPCR Reaction Buffer	17 μL	561 μL
E7-110 Primer&Probe MIX	3 μL	99 μL
Total volume	20 μL	660 μL
Component	**Sample volume (1 well)**	**Sample volume (30 wells) ***
qPCR Reaction Buffer	17 μL	561 μL
E7-240 Primer&Probe MIX	3 μL	99 μL
Total volume	20 μL	660 μL

*Includes 10% loss redundancy for 30 wells.

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

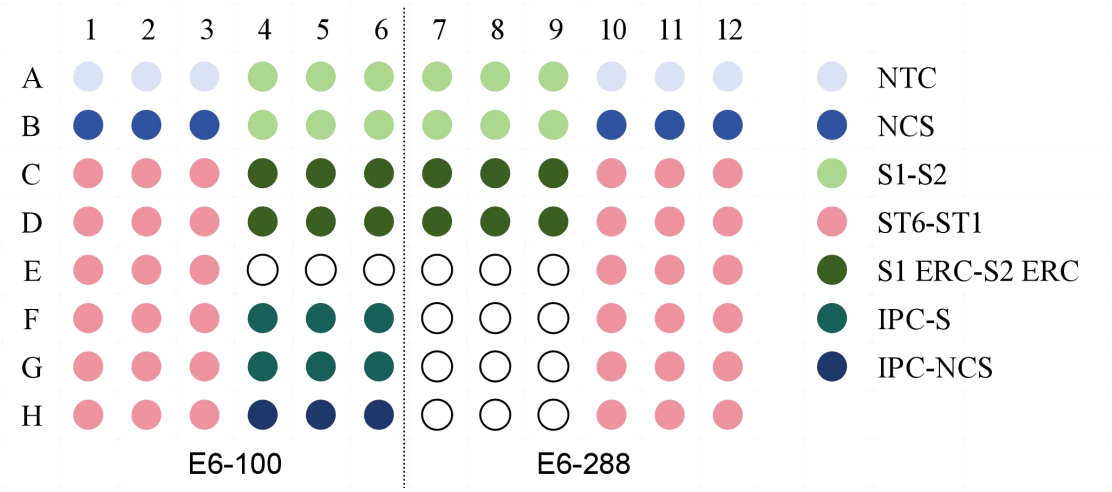
IPC Reaction MIX preparation

Component	Sample volume (1 well)	Sample volume (30 wells) *
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

*Includes 10% loss redundancy for 30 wells.

Note: IPC for NCS and Test samples are required for each test.

3. Dispense the prepared qPCR MIX& IPC MIX into the 96-well plate according to the plate layout below (example of E6-100 and E6-288). Add No Template Control (NTC, use DDB as the NTC in this kit), NCS, Samples, ERC, IPC-NCS, IPC-Samples, and Standards into the corresponding wells. The number of samples and plate layout can be adjusted depending on the actual testing requirement.



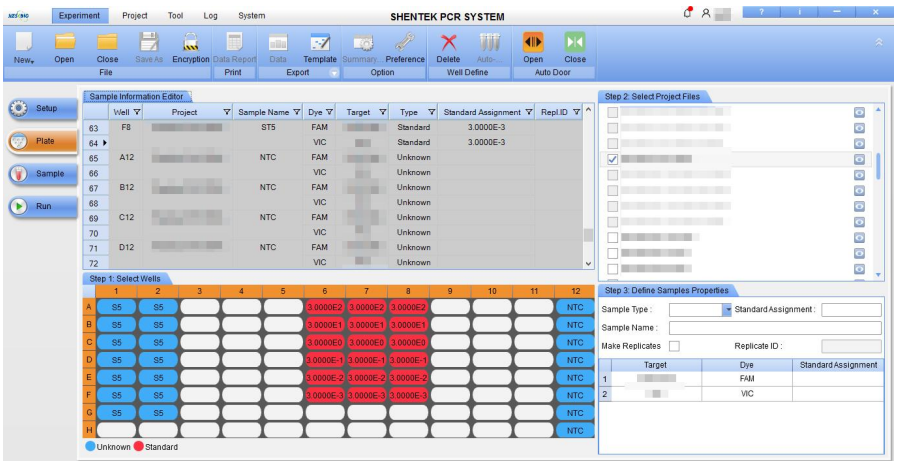
a) Add 20 µL of qPCR MIX & IPC MIX (prepared on step 2) to each colored reaction well.

b) Sequentially add 10 µL of the following to the corresponding wells: NTC, NCS, Samples (S1-S2), ERC (S1 ERC-S2 ERC), IPC-NCS, IPC-Samples, and serial diluted Standard (ST6-ST1).

4. Close the tube lids or seal the plate with sealing film carefully. Gently vortex mix and briefly centrifuge for 10 seconds.

qPCR program setting

◆The following experiment program setup procedures applies to SHENTEK-96H Real-time PCR System, software Version 9.0 example.



1. Click on “Experiment” and select “Setup”
2. Select Type “Qualitative/Absolute Quantitative” in the Experiment Information.
3. Enter the "Plate" page.
4. Select Step 1: Select wells.
5. Select Step 2: Select Project Files – “HPV Residual DNA ”and “HPV IPC”from list.
6. Select Step 3: Define Samples Properties, enter sample information.
7. Click "Start" to run the program on the "Run" page.

◆For other quantitative PCR systems, the program setup is as follows (Refer to the applicable instrument or software documentation.):

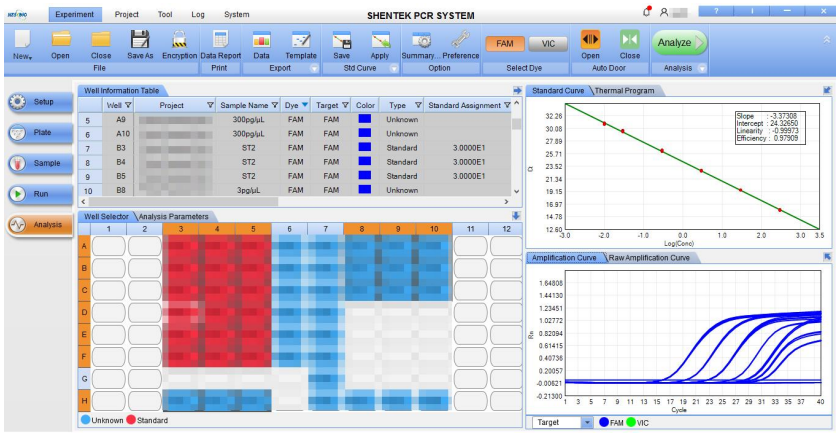
1. Create a new blank program and select the absolute quantification detection template.
2. Create a new detection probe, name it as “HPV E6 DNA-100”, define the reporting fluorophore as FAM, the quenching fluorophore as none, and the reference fluorophore as ROX (optional).
3.Create a new detection probe, name it as “HPV IPC”, define the reporting fluorophore as VIC, the quenching fluorophore as none
4.Creat new detection probe for E6-288, E7-110 and E7-240,separately as step2.
5. Set up the reaction program:

Temperature	Duration (mm: sec)	Cycle number
95°C	10: 00	1
95°C	00: 15	40
60°C*	01: 00	

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

Results analysis

◆For SHENTEK-96H Real-time Fluorescent PCR System, software Version 9.0:



1. Enter the "Analysis" page, click the “Analyze” icon to check the slope, intercept, Linearity, and amplification efficiency of the standard curve.
2. In the "Well Information Table", the values for each reaction well can be displayed in units of "copies/μL".

◆ For ABI 7500 Real-Time PCR System, software Version 1.5.1:

1. Enter the "Results" section and check the Amplification Plot panel. Set the Threshold to "0.02" and the Baseline to "Auto". Click "Analyze" to preliminarily check the amplification curve for normality.
2. In the "Results" section, under the Plate panel, set the Task field for the standard curve wells to "Standard". Name the corresponding wells from ST1 to ST6, and assign values based on serial dilutions table.
3. In the "Results" section, under the Plate panel, set the Task field for the NTC, NCS, Sample, IPC-NCS, IPC-Sample, and ERC to "Unknown". Name the corresponding Sample Names as NTC, NCS, S, IPC-NCS, IPC-S, and ERC. Then click "Analyze."
4. In the "Results" section under the Standard Curve panel, the slope, intercept, and R^2 values for the standard curve can be viewed.
5. In Report panel, the Mean Quantity field displays the detection values for NTC, NCS, ERC, IPC-NCS, IPC-S, and test samples, with units in copies/μL.

Note: The parameter settings listed above are generally recommended for result analysis and could be adjusted depending on the specific instrument model and software version. In most cases, the instrument performs the interpretation automatically.

Interpretation of results

1. Adding standard recovery calculation based on the detection results of the test samples and the ERC Sample, the recovery must fall within the range of 50% to 150%.
2. 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.
3. If the verified quantitation limit (QL) concentration is lower than the lowest concentration of the standard curve, the NCS detection value should be lower than the quantitation limit (QL) concentration.

Revision	Date	Description
A/0	28 May 2025	Basic Version of User Guide.
A/1	20 Aug. 2025	Update Table 2.
A/2	26 Aug. 2026	Include the RUO statement and change storage instructions.

Effective Date: 26 Aug. 2026

■ Support & Contact

Huzhou Shenke Biotechnology Co., Ltd.
www.shentekbio.com
Address: 8th Floor, 6B Building, No.1366 Hongfeng Road, Huzhou 313000, Zhejiang Province, China
E-mail: info@shentekbio.com
Phone: +1 (908) 822-3199 / (+86) 400-878-2189