resDetect™ Vero resDNA Quantitation Kit (qPCR)

Catalog Number：OPA-R018

Assay Tests: 100 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

IMPORTANT: Please carefully read this user guide before performing the experiment.

Product information

Vero resDNA Quantitation Kit is designed for quantitative detection of residual Vero DNA in biopharmaceutical productions (Vaccines). Use the kit after extracting host cell DNA from test samples. For achieving the better DNA recovery, it is recommended to use the **resDetect™ resDNA Sample Preparation Kit II (Magnetic Beads) (Cat. No. OPA-R024)** in combination.

Residual Vero DNA is quantified using a real-time polymerase chain reaction (PCR) assay. The PCR-based assay is sensitive and specific for DNA from Vero genome and not subject to detection of human or environmental DNA that might be introduced during sample handling.

To generate the standard curve used to quantitate the DNA in test samples, the Vero assays require dilutions. Control DNA for standard curve generation is included in the kits. Linearity is demonstrated by analysis of standard DNA from Vero ranging from **3 fg/μL~30 pg/μL.** Limit of Detection is 1 fg/μL.

Contents and Storage

The kit contains sufficient reagents to run 100 PCR reactions each with a final reaction volume of 30 μL.

Contents	Colors	Amount	Storage
2×qPCR Master Mix		1.0mL×2	-30℃ to -15℃ **Note:** Primer＆Probe Mix and 2×qPCR Master Mix need protect from light.
Vero Primer＆Probe Mix		700μL×1	
Vero DNA Control (3 ng/μL)		100μL×1	
Dilution Buffer		1.5mL×3	
DNase/RNase-Free Water		1.0mL×1	

The unopened kit is stable for 18 months from the date of manufacture if stored at -30°C to -15°C.

Required materials not supplied.

Instrument	Real-time PCR instrumentation
Consumables	96-Well Reaction Plate, Covers
	Nuclease-free, DNA-free aerosol-resistant pipet tips
	Low DNA-Binding Microcentrifuge Tubes (Nuclease-free, DNA-free) to prepare working solution, dilutions, and mixes

Workflow

Prepare the DNA control serial dilutions for the standard curve

Preparation and extraction of NEC or ERC (Optional)

Prepare the PCR reaction mix

Create the plate document and run the plate

Analyze the results

NOTE: NEC=Negative Extraction Control； ERC=Extraction/Recovery Control

Prepare the DNA control serial dilutions for the standard curve.

Guidelines for standard dilutions[Y1]

- Prepare the standard curve and the test samples in different areas of the lab.
- Use Low DNA-Binding microcentrifuge tubes and different sets of pipettors for test sample preparation and for standard curve preparation and aliquoting to avoid cross-contamination of test samples.
- Vortex each tube for **20-30 seconds** to ensure thorough mixing of the contents before proceeding with each dilution step.
- Briefly centrifuge to collect all the liquid at the bottom before making the next dilution.

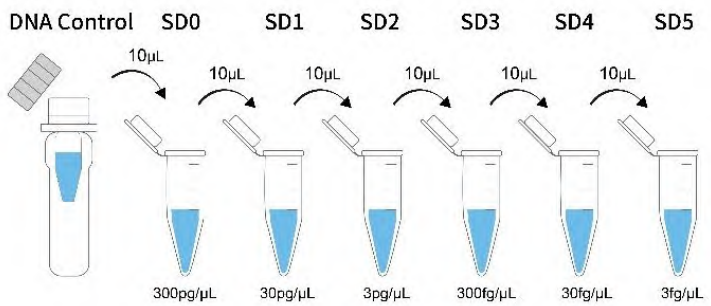
Prepare the control serial dilutions

1. Label low DNA-binding microfuge tubes: **SD0, SD1, SD2, SD3, SD4, SD5, NTC Template**, where **SD** indicates serial dilutions and **NTC** indicates the no template control.
2. Add **30-40 µL** of DNase/RNase-Free Water to tube **NTC Template**. Put aside.
3. Add **90 µL** of Dilution Buffer to tubes **SD0, SD1, SD2, SD3, SD4, SD5**.
4. Remove the tube of Vero DNA control (3 ng/µL) from the freezer.
5. After the DNA thaws, vortex it gently for 20-30 seconds, then briefly centrifuge to collect the solution at the bottom.
6. Perform the serial dilutions:

   a. Add **10 µL** of the DNA control to the tube that is labeled **SD0**, then vortex thoroughly and briefly centrifuge.

   b. Transfer **10 µL** of the DNA from tube **SD0** to tube **SD1**, then vortex thoroughly and briefly centrifuge.

   c. Continue to transfer **10 µL** of DNA from the previous dilution tube to the next dilution tube until you add DNA to tube **SD5**. After each transfer, vortex thoroughly, then centrifuge briefly. Process of dilutions is shown in the following figure.
7. Store the **SD** tubes at 4°C for use within 24 hours.



Preparation and extraction of NEC and ERC (Optional)

Preparation and extraction of NEC

A Negative Extraction Control (NEC) omits any DNA template from a reaction. This control is used to monitor contamination during nucleic acid extraction. In cases where large numbers of DNA samples need extracted, it is recommended that negative extraction controls are included between the samples for testing.

1. Label low DNA-binding 1.5 mL microfuge tubes “NEC”.
2. Add **100 µL** of 1×PBS (free of Mg^{2+} and Ca^{2+}) or 1×TE (pH7.0~pH8.0) to each tube.

When finished, extract DNA from the tubes according to the resDNA Sample Preparation Kit II User Guide (Cat. No. OPA-R024), then quantify the extracted DNA in each tube using this Kit (Cat. No. OPA-R018) as described in the following section.

Preparation of Extraction/Recovery Control (ERC) (Optional)

Extraction/Recovery Control (ERC) can be used to assess the efficiency of DNA extraction, recovery, and quantitation from test samples. Additionally, ERC can be used to verify assay and system performance. The following procedure describes the preparation of ERC sample containing Vero DNA control per well for qPCR analysis.

1. For each sample, label low DNA binding 1.5 mL microfuge tubes “ERC”.
2. Add the appropriate volume of test sample to each tube.
3. Add **20 µL** of DNA from tube **SD3 (300 fg/µL)** to each ERC tube, then vortex gently.

When finished, extract DNA from the tubes according to the resDNA Sample Preparation Kit II User Guide (Cat. No. OPA-R024), then quantify the extracted DNA in each tube using this Kit (Cat. No OPA-R018) as described in the following section.

Note: Adjust the amount of Vero DNA control added to the sample for those test samples that contain higher background levels of DNA. To ensure accurate results, the amount of Vero DNA control added to a test sample should be approximately two to three times the amount of DNA measured in the test sample without the addition of the Vero DNA control. To calculate the efficiency of DNA recovery and quantitation from the test samples, subtract the amount of DNA measured in the sample without the addition of Vero DNA control from the amount of DNA measured in the ERC sample.

Prepare the PCR reaction mix

Prepare serial dilutions of Vero DNA control from the same experiment to create a standard curve and to determine sample recovery rate.

1. Determine the number of controls and test samples whose DNA content you will quantify. Number of reaction wells is equal to three times the sum of **NTC, NEC, ERC, SD1-SD5, and test samples**.
2. Thaw reagents completely on ice, thoroughly mix reagent, and briefly centrifuge. Prepare a 2.0 mL tube for **Working Mix** (not add DNA template) using the reagents and volumes shown in the table below, thoroughly mix reagent, and briefly centrifuge. **IMPORTANT!** To compensate for pipetting losses, it is recommended that the **N** ~~is~~ be at least number of reaction wells plus 2 or 3.

Kit Reagents	Volume for 1 reaction (30 µL)	Volume for Working Mix
2×qPCR Master Mix	15 µL	15 µL×**N**
Vero Primer＆Probe Mix	5 µL	5 µL×**N**
DNA template	10µL	Add DNA template to each well separately, not as part of Working Mix
Total	30 µL	20 µL×**N**

3. Add **20 µL** Working mix to each well separately.
4. Add **10 µL** DNA template to the corresponding wells. **Final volume** of PCR reaction is **30 µL.** It is recommended that the above DNA samples (test samples, NTC, NEC,

ERC, and SD) should be placed in different zones during the design and layout of the reaction wells to avoid cross contamination and inaccurate test results.

NOTE: Set up a 96-well PCR plate using the example plate layout shown in the following page.

5. Seal the plate with an optical film, then quick-spin with a centrifuge rotor that is compatible with 96-well plates.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A									SD1	SD1	SD1	
B	S1	S1	S1		S1(ERC)	S1(ERC)	S1(ERC)		SD2	SD2	SD2	
C	S2	S2	S2		S2(ERC)	S2(ERC)	S2(ERC)		SD3	SD3	SD3	
D	S3	S3	S3		S3(ERC)	S3(ERC)	S3(ERC)		SD4	SD4	SD4	
E									SD5	SD5	SD5	
F												
G	NEC	NEC	NEC									
H									NTC	NTC	NTC	

S=Sample ; NTC=No Template Control ; NEC=Negative Extraction Control ; ERC=Extraction/Recovery Control

Note: The plate layout is a suggested plate layout. Adjust the layout according to the number of test samples to be run.

Create the plate document and run the plate

The following instructions apply only to the ABI 7500 instrument. If you use a different instrument, refer to the applicable instrument guide for setup guidelines.

1. Create a new experiment, enter the experiment name in the Plate name field.
2. Select the **Quantitation Standard Curve** mode, **TaqMan** reagents, and **Standard** mode.
3. In the Plate Setup, enter the Target Name. Select **FAM** in the Reporter Dye drop-down list. Select **(None)** in the Quencher Dye drop-down list. Select **ROX** in the Passive Reference Dye drop-down list.
4. Set up the standard curve as shown in the Plate Layout. Assign the tasks and the enter the appropriate Quantity for each set of triplicates. (SD1-SD5, 30000, 3000, 300, 30, 3 fg/μL)
5. Set up the test samples and controls as shown in the Plate Layout.
6. Set up the qPCR reaction program according to following Table.
7. Select the reaction volume to 30 μL, click "Start Run" in the Run interface to start the qPCR run, and analyze the results after completion.

Step	Temperature	Cycles	Time	Signal Collection
UDG	37℃	1×	2 mins	No
Initial Denaturation	95℃	1×	5 mins	No
Denaturation	95℃	40×	10s	No
Extension	60℃		30s	**Yes**

Analyze the results

After the qPCR run is finished, use the general procedure to analyze the results. The threshold value for ABI7500 is set to 0.4, and it uses auto baseline; For other instruments, the setting of parameters should be adjusted according to the specific instrument user guide and software version.

For more information, refer the Getting Started Guide that is supplied with the specific analysis software. The acceptance criteria of results are shown in the following list:

1. The Standard curve: $R^2 \geqslant 0.98$, Eff%=90-110%.
2. The detection result of NTC should be undetermined or Ct value > 35.
3. The Ct value of NEC should be greater than the Ct value of the SD5.
4. Ct values should be consistent with replicates, the differences between the Ct values of replicates is less than 0.5.
5. The spike recoveries of test samples and controls should be between 50%-150%.

Note: Calculate the concentration of the test sample (pg/μL or fg/μL) based on the standard curve, the Ct value of test sample is only valid for concentration calculation within the assay range of standard curve. When Ct values is outside the range of standard curve, do not use the data to calculate the concentration of test sample.