resDetect™ Plasmid resDNA Quantitation Kit (qPCR)

Catalog Number： OPA-R009

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 your experiment.

Contents

Product Information

The Plasmid resDNA Quantitation Kit is designed for quantitative detection of residual plasmid DNA in biopharmaceutical productions (Antibodies, cells, viruses, etc.). To achieve better DNA recovery, it is recommended to use the **resDetect™ resDNA Sample Preparation Kit (Magnetic Beads) (Cat. No. OPA-R005)** in combination with this kit. For more information about the extraction kit, refer to the resDNA Sample Preparation Kit User Guide (ACROBiosystems.com).

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

Linearize control DNA and circular control DNA for standard curve generation are included in this kit. Select the DNA control according to your experiment requirement.

Detection range of Linearize DNA Control: **4×10^{1} copies/μL~4×10^{6} copies/μL**.

Detection range of Circular DNA Control: **2×10^{2} copies/μL~2×10^{6} copies/μL**.

Plasmid copy number (copies/μL) = $6.02\times10^{14}\times$Plasmid concentration(ng/μL)/(Plasmid bases number×660).

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Contents and Storage

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

Contents	Amount	Storage
Plasmid Primer & Probe Mix	550 μL×1	-30℃ to -15℃ **Note:** 2×qPCR Master Mix and Primer＆Probe Mix need protection from light.
2×qPCR Master Mix	1.6 mL×1	
Circular DNA Control (2×10^8 copies/μL)	50 μL×1	
Linearize DNA Control (4×10^8 copies/μL)	50 μL×1	
DNA Dilution Buffer	1.5 mL×3	

The unopened kit can be stored stably for 18 months from the date of manufacture when kept at a storage temperature between -30°C and -15°C.

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Required materials not supplied

Equipment	Real-time PCR instrumentation
Reagent	DNase/RNase-Free Water
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.

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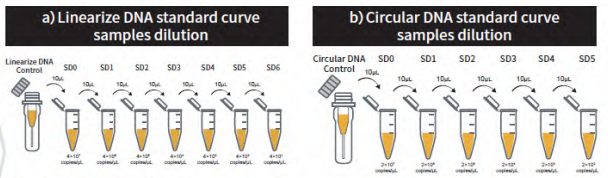
Workflow

Step 1 Prepare the kit reagent and standard curve dilution

2×qPCR Master Mix
Primer & Probe Mix
Circular DNA Control
Linearize DNA Control
DNA Dilution Buffer

for 5~10s
Briefly centrifuge



Preparation of ERC: Taking a spiking amount of 8×10^4 copies linearize DNA as an example, add 20 µL of SD4 (4×10^3 copies/µL) to 100 µL of the sample to be tested, vortex it thoroughly, and this is the ERC.

Note: ***The SD4 used for spiking in the ERC should be from the same tube as the SD4 in the standard curve samples.***

Step 2 Prepare the PCR reaction mix

Working Mix(for 1 reaction)
+15 µL 2×qPCR Master Mix (uncolored cap)
+5 µL Primer & Probe Mix (brown cap)

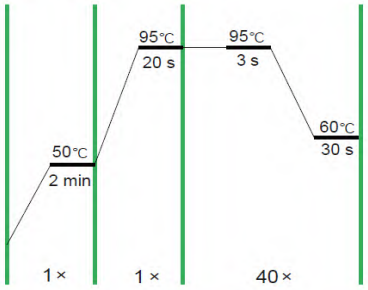
+20 µL Working mix

Step 3 Add samples and controls

Sample (+10 µL Sample purified solution)
ERC (+10 µL ERC purified solution)
NEC (+10 µL NEC purified solution)
NTC (+10 µL DNase/RNase-Free Water)

Close lid tightly.
for 30 s

Step 4 Create the plate document and run qPCR



+ add vortex centrifuge

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Protocol

Prepare the DNA control serial dilutions for the standard curve

1. Guidelines for standard dilutions

1.1 Prepare the standard curve and the test samples in different areas of the lab.

1.2 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.

1.3 Vortex each tube for **5-10 seconds** to ensure thorough mixing of the contents before proceeding with each dilution step.

1.4 Briefly centrifuge to collect all the liquid at the bottom before making the next dilution.

2. Prepare the linearize DNA control serial dilutions (Optional)

2.1 Label low DNA-binding microfuge tubes: **SD0, SD1, SD2, SD3, SD4, SD5, SD6**, **NTC**, where **SD** indicates serial dilutions and **NTC** indicates the no template control.

2.2 Add **30-40 µL** of DNase/RNase-Free Water to tube **NTC**. Put aside.

2.3 Add **90 µL** of DNA Dilution Buffer to tubes **SD0, SD1, SD2, SD3, SD4, SD5, SD6.**

2.4 Remove the tube of Linearize DNA control (4×10^8 copies/µL) from the freezer.

2.5 After the DNA thaws, vortex it gently for 5-10 seconds, then briefly centrifuge to collect the solution at the bottom.

2.6 Perform the serial dilutions:

a. Add **10 µL** of the Linearize 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 **SD6**. After each transfer, vortex thoroughly, then centrifuge briefly. The process of dilutions is shown in the following figure 1.

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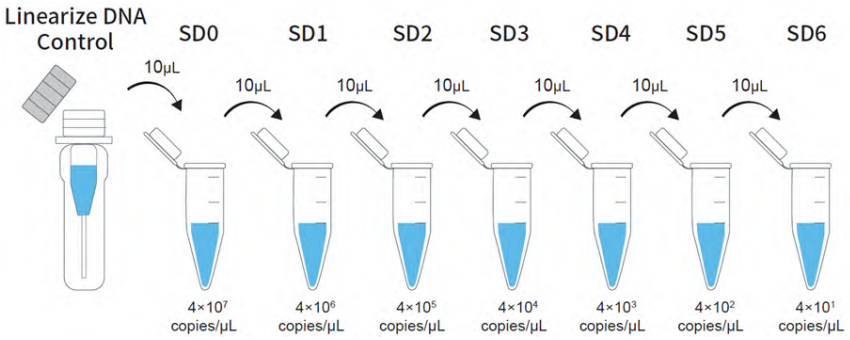

Figure 1. Linearize DNA Control Dilution Diagram

2.7 Store the **SD** tubes at 4°C for use within 24 hours.

3. Prepare the circular DNA control serial dilutions (Optional)

3.1 Label low DNA-binding microfuge tubes: **SD0, SD1, SD2, SD3, SD4, SD5**, **NTC**, where **SD** indicates serial dilutions and **NTC** indicates the no template control.

3.2 Add **30-40 µL** of DNase/RNase-Free Water to tube **NTC**. Put aside.

3.3 Add **90 µL** of DNA Dilution Buffer to tubes **SD0, SD1, SD2, SD3, SD4, SD5.**

3.4 Remove the tube of Circular DNA Control (2×10^8 copies/µL) from the freezer.

3.5 After the DNA thaws, vortex it gently for 5-10 seconds, then briefly centrifuge to collect the solution at the bottom.

3.6 Perform the serial dilutions:

a. Add **10 µL** of the Circular 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 2.

3.7 Store the **SD** tubes at 4°C for use within 24 hours.

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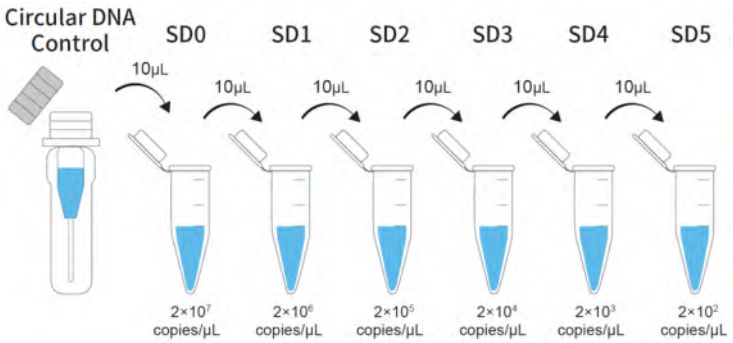

Figure 2. Circular DNA Control Dilution Diagram

Preparation and extraction of NEC and ERC (Optional)

1. Preparation and extraction of Negative Extraction Control (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.

1.1 Label low DNA-binding 1.5 mL microfuge tubes "NEC".

1.2 Add **100 µL** of 1×PBS (free of Mg^{2+} and Ca^{2+}) or 1×TE (pH7.0~pH8.0) to each tube.

2. Preparation and extraction of Extraction/Recovery Control (ERC)(Optional)

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

2.1 For each sample, label low DNA binding 1.5 mL microfuge tubes "ERC".

2.2 Add the appropriate volume of test sample to each tube.

2.3 Add **20 µL** of DNA from tube **SD4 ($4×10^3$ copies/µL)** to each ERC tube, then vortex gently.

NOTE: Adjust the amount of linearize DNA control added to the sample for those test samples that exhibit higher background DNA levels. To ensure accurate results, the amount of linearize DNA control added to a test sample should be approximately two to three times the quantity of DNA

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measured in the test sample prior to the addition of the linearize DNA control. To calculate the efficiency of DNA recovery and quantitation from the test samples, subtract the amount of DNA measured in the sample before adding linearize DNA control from the amount of DNA measured in the ERC sample.

When the circular DNA control is selected as the standard curve, use one of the standard curve samples to prepare ERC.

When NEC and ERC preparations are finished, extract DNA from the tubes according to the resDNA Sample Preparation Kit User Guide (Cat. No. OPA-R005), then quantify the extracted DNA in each tube using this kit (Cat. No. OPA-R009) as described in the following section.

Prepare the PCR reaction mix

Prepare serial dilutions of plasmid 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. The number of reaction wells equals three times the sum of **NTC, NEC, ERC, SDs and test samples.**
2. Thaw reagents completely on ice, thoroughly mix reagents, 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** equals the 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**
Plasmid Primer＆Probe Mix	5 μL	5 μL×**N**
DNA template	10 μL	Add DNA template to each well separately, not as part of

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		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 SDs) 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									**SD6**	**SD6**	**SD6**	
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. There is no SD6 for circular DNA standard curve.

Create the plate document and run the plate

The following instructions apply only to the ABI 7500 instrument. If you use a different

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instrument, refer to the applicable instrument guide for setup guidelines.

1. Create a new experiment, and 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.
   a. When use the Linearize DNA Control, assign the tasks and the enter the appropriate Quantity for each set of triplicates. (SD1-SD6: 4×10^6, 4×10^5, 4×10^4, 4×10^3, 4×10^2, 4×10^1 copies/μL).
   b. When use the circular DNA Control, assign the tasks and the enter the appropriate Quantity for each set of triplicates. (SD1-SD5: 2×10^6, 2×10^5, 2×10^4, 2×10^3, 2×10^2 copies/μL).
5. Set up the test samples and controls as shown in the Plate Layout.
6. Set up the qPCR reaction program according to the following Table.
7. Set 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
1	50℃	1×	2 min	No
2	95℃	1×	20 s	No
3	95℃	40×	3 s	No
	60℃		30 s	**Yes**

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Analyze the results

After the qPCR run is finished, use the general procedure to analyze the results. For more information, refer the Getting Started Guide that is supplied with the specific analysis software. The acceptance criteria of results are shown as follows:

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 SD6.
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%. The calculation formula of spiked recovery rate is as follows:

$$\text{Spiked Recovery Rate} = \frac{\text{the detected amount of ERC (copies)} - \text{the detected amount of un-spiked sample (copies)}}{\text{Theoretical spiked amount (copies)}} \times 100\%$$

The calculation formula can be more detailed as below:

$$\text{Spiked Recovery Rate} = \frac{\text{The detected concentration of ERC (spiked sample) (copies/μL)} \times \text{Volume of eluted DNA} - \text{The detected concentration of un-spiked sample (copies/μL)} \times \text{Volume of eluted DNA (μL)}}{\text{Theoretical spiked concentration (copies/μL)} \times \text{Spiked volume (μL)}} \times 100\%$$

NOTE: Calculate the concentration of the test sample 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 value is outside the range of standard curve, do not use the data to calculate the concentration of test sample.

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