



resDetect™ E1A resDNA Quantitation Kit (qPCR)

Catalog Number: OPA-R008

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

E1A resDNA Quantitation Kit is designed for quantitative detection of residual E1A DNA in biopharmaceutical productions (Antibodies, cells, viruses, etc.). Use the kit after you extract host cell DNA from test samples. For extraction information, see the resDNA Sample Preparation Kit User Guide (Cat. No. OPA-R005)

Residual E1A DNA is quantified using a real-time polymerase chain reaction (PCR) assay. The PCR-based assay is sensitive and specific for DNA from E1A gene 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 E1A assays require six dilutions. Control DNA for standard curve generation is included in the kits. Linearity is demonstrated by analysis of standard DNA from E1A ranging from 40 copies/ μL ~ 4×10^6 copies/ μL .

Plasmid copy number (copies/ μL) = $6.02 \times 10^{14} \times \text{Plasmid concentration}(\text{ng}/\mu\text{L}) / (\text{Plasmid bases number} \times 660)$.

Contents and Storage

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

Contents	Amount	Storage
2 \times qPCR Master Mix	1.6 mL \times 1	-30°C to -15°C Note: Primer & Probe Mix need protect from light.
E1A Primer & Probe Mix	550 μ L \times 1	
Linearize DNA Control (4 \times 10 ⁸ copies/ μ L)	50 μ L \times 1	
DNA Dilution Buffer	1.5 mL \times 3	

The unopened kit is stable for 12 months from the date of manufacture if stored at -30°C to -15°C.

Required materials not supplied.

Instrument	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

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

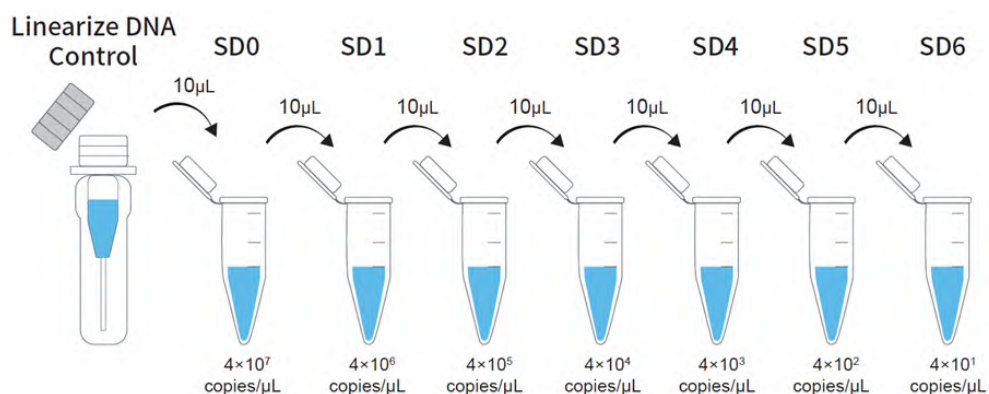
Prepare the DNA control serial dilutions for the standard curve.

Guidelines for standard dilutions

- 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 **5-10 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, SD6, NTC**, 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**. Put aside.
3. Add **90 µL** of DNA Dilution Buffer to tubes **SD0, SD1, SD2, SD3, SD4, SD5, SD6**.
4. Remove the tube of Linearize DNA Control (4×10^8 copies/µL) from the freezer.
5. After the DNA thaws, vortex it gently for 5-10 seconds, then briefly centrifuge to collect the solution at the bottom.
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. 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 1X PBS (free of Mg²⁺ and Ca²⁺) or 1×TE (pH7.0~pH8.0) to each tube.

Preparation and extraction of ERC

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 E1A Linearize 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 **SD4 (4×10³ 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 contain higher background levels of DNA. To ensure accurate results, the amount of Linearize DNA control that you add 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 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 without the addition of Linearize DNA control from the amount of DNA measured in the ERC sample.

When NEC and NRC 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-R008) as described in the following section.

Prepare the PCR reaction mix

Prepare serial dilutions of E1A Linearize 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-SD6, 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 equals to number of reaction wells plus 2 or 3.

Kit Reagents	Volume for 1 reaction (30 μ L)	Volume for Working Mix
2 \times qPCR Master Mix	15 μ L	15 μ L \times N
E1A Primer & Probe Mix	5 μ L	5 μ L \times N
DNA template	10 μ L	Add DNA template to each well separately, not as part of Working Mix
Total	30 μ L	20 μ L \times N

3. Add **20 μ L** Working mix to each well separately.
4. Add **10 μ L** DNA template to the corresponding wells. **Final volume** of PCR reaction is **30 μ L**. 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									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.

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-SD6, 4×10^6 , 4×10^5 , 4×10^4 , 4×10^3 , 4×10^2 , 4×10^1 copies/ μ L)
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 μ L, 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	50°C	1×	2 min	No
Initial Denaturation	95°C	1×	20 sec	No
Denaturation	95°C	40×	3 sec	No
Extension	60°C		30 sec	Yes

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 in the following list:

1. The Standard curve: $R^2 \geq 0.98$, Eff%=90-110%.
2. The determination of NTC detection results should be involve Ct value >35 or Undetermined.
3. The spike recoveries of test samples and controls should be between 50%-150%.

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 values is outside the range of standard curve, do not use the data to calculate the concentration of test sample.