



# resDetect™ HEK293T resDNA Ultra-Low Quantitation Kit (qPCR)

## User Manual V1.0

**Catalog Number:** OPA-R032

**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.**

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## Product Information

The resDetect™ HEK293T resDNA Ultra-Low Quantitation Kit (qPCR) is designed for the quantitative detection of residual HEK293T DNA in biopharmaceutical production (e.g., antibodies). For optimal DNA recovery, it is recommended to use the resDetect™ HEK293 /HEK293T resDNA Sample Preparation Kit (Magnetic Beads) (Cat. No.: OPA-R033) in conjunction with this kit. For additional information on the extraction kit, refer to the resDetect™ HEK293 /HEK293T resDNA Sample Preparation Kit User Guide (available at [ACROBiosystems.com](http://ACROBiosystems.com)).






Residual HEK293T DNA is quantified using a real-time polymerase chain reaction (PCR) assay. The PCR-based assay is both sensitive and specific to HEK293T genomic DNA and does not detect environmental DNA that may be introduced during sample handling. The kit was developed to meet the sensitivity requirements defined by the WHO (10 ng HEK293T DNA per therapeutic dose).

To generate the standard curve for DNA quantification in test samples, six dilutions are required for the HEK293T assay. Control DNA for standard curve generation is provided in the kit. Linearity is demonstrated by analyzing standard HEK293T DNA concentrations ranging from 3 fg/μL to 300 pg/μL. The limit of quantitation (LOQ) is 3 fg/μL.

## Contents and Storage

The kit contains sufficient reagents to perform up to 100 PCR reactions, each with a final reaction volume of 30  $\mu$ L.

Table 1. Components

Contents	Colors	Amount	Storage
2 $\times$ qPCR Master Mix		1.0 mL $\times$ 2	-30°C to -15°C <b>Note:</b> 2 $\times$ qPCR Master Mix and Primer & Probe Mix need protection from light.
HEK293T Primer & Probe Mix		700 $\mu$ L $\times$ 1	
HEK293T DNA Control (3 ng/ $\mu$ L)		100 $\mu$ L $\times$ 1	
Dilution Buffer		1.5 mL $\times$ 3	
DNase/RNase-Free Water		1.0 mL $\times$ 1	

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

## Required materials not supplied

Table 2. Required Materials

Category	Item	Experimental Recommendation
Equipment	Real-Time PCR instrument	FAM Channel
	Vortex	2500~3000 rpm
	Mini centrifuge	3000~6000 rpm
	96-well plate centrifuge	3000~6000 rpm
	Pipettes	1000 $\mu$ L, 100 $\mu$ L, 20 $\mu$ L, and 10 $\mu$ L
Consumables	96-well PCR plate, sealing films	For qPCR instruments, nuclease-free, DNA-free.
	Pipet tips	Nuclease-free, DNA-free, with filter.
	Low DNA-Binding Microcentrifuge Tubes	Nuclease-free, DNA-free.

## Compatible Real-Time PCR System (including but not limited to)

- ABI 7500/7500 Fast Real-Time PCR System
- ABI QuantStudio<sup>®</sup> 5 Real-Time PCR System
- Roche Light Cycler 480 II
- Bio-Rad CFX96 Real-Time PCR System
- Bioer LineGene 9600 Plus Real-Time PCR System
- Hongshi SLAN-96S Real-Time PCR System






## List of Abbreviations



Table 3. Abbreviation


Abbreviations	Full Name
HEK293	Human Embryonic Kidney 293
Ct	Cycle Threshold
NTC	No Template Control
ERC	Extraction/Recovery Control
NEC	Negative Extraction Control
SERC	Sample Extraction/Recovery Control

## Workflow

### Step 1 Prepare the kit reagent and standard curve dilution

-  2×qPCR Master Mix
-  Primer & Probe Mix
-  DNA Control
-  Dilution Buffer
-  DNase/RNase-Free Water

 for 20~30s  
 Briefly centrifuge



**Preparation of ERC:** Taking a spiking amount of 6 pg as an example, add 20 μL of SD4 (300 fg/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


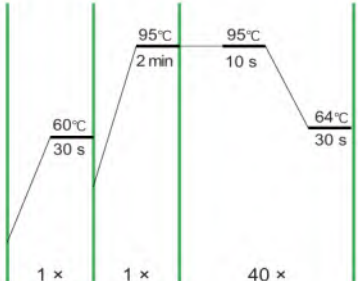
### Step 3 Add samples and controls





Close lid tightly.

 for 30 s

### Step 4 Create the plate document and run qPCR

+ add
 vortex
 centrifuge

## Protocol

### Preparation of DNA control Serial Dilutions for Standard Curve

Prepare serial dilutions of HEK293T DNA control for the same experiment to generate a standard curve and determine the sample recovery rate.

#### 1. Guidelines for Standard Dilutions

- 1.1 Prepare the standard curve and test samples in separate areas of the laboratory.
- 1.2 Use low DNA-binding microcentrifuge tubes and dedicate separate sets of pipettors for standard curve preparation and test sample handling to prevent cross-contamination.
- 1.3 Vortex each tube for **10-20 seconds** to ensure thorough mixing before proceeding to the next dilution step.
- 1.4 Briefly centrifuge each tube to collect all liquid at the bottom prior to the next dilution.

#### 2. Preparation of Control Serial Dilutions

- 2.1 Label the microfuge tubes: **SD1, SD2, SD3, SD4, SD5, SD6, NTC**. The **SD** indicates serial dilutions and **NTC** indicates the no template control.
- 2.2 Prepare the NTC tube: Add **30-40 µL** of DNase/RNase-Free Water to the **NTC** tube. Set the NTC tube aside, it will be used later as the template of NTC.
- 2.3 Add **90 µL** of Dilution Buffer to each of the tubes **SD1, SD2, SD3, SD4, SD5, and SD6**.
- 2.4 Remove the HEK293T DNA control tube (3 ng/µL) from the freezer.
- 2.5 Allow the DNA to thaw completely. Gently vortex the tube for 10-20 seconds, then briefly centrifuge to collect the solution at the bottom.
- 2.6 Perform the serial dilutions:
  - a. Add **10 µL** of the DNA control to the tube labeled **SD1**. Vortex thoroughly and briefly centrifuge.
  - b. Transfer **10 µL** of DNA from **SD1** to **SD2**. Vortex thoroughly and briefly centrifuge.
  - c. Repeat the transfer process sequentially from each previous dilution tube to the next. After each transfer, vortex thoroughly and centrifuge briefly. The dilution process is illustrated in the following figure.

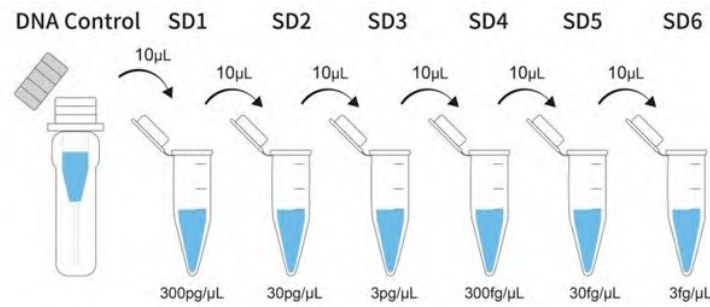


Figure 1. DNA Control Dilution Diagram

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

**NOTE:** This kit enables quantification using six standard concentrations (SD1–SD6) for standard curve setup, supporting both conventional quantification mode (SD1–SD5) and high-sensitivity quantification mode (SD1–SD6), which can be flexibly selected based on the required limit of detection (LOD).

### Preparation of the PCR Reaction Mix

1. Determine the number of controls and test samples to be quantified. The total number of reaction wells is three times the sum of **NTC**, **NEC**, **ERC**, **SERC**, **SD1-SD6**, and **test samples**.
2. Thaw all reagents completely on ice. Thoroughly mix each reagent and briefly centrifuge. Prepare a 2.0 mL tube for the **Working Mix** (without adding DNA template) using the reagents and volumes listed in the table below. Mix thoroughly and briefly centrifuge.

**IMPORTANT!** To compensate for pipetting losses, it is recommended that the **N** equals to the number of reaction wells plus 2 or 3.

Table 4. qPCR Working Mix Preparation

Reagents	Volume/Reaction	Volume for Working Mix
2×qPCR Master Mix	15 µL	15 µL×N
HEK293T Primer&Probe Mix	5 µL	5 µL×N
<b>Total</b>	30 µL	20 µL×N

3. Design the plate layout by allocating test samples, NEC, ERC and SD to separate zones to prevent cross-contamination and ensure accurate test results. Below is an example of the plate layout.

Table 5. Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A									SD1	SD1	SD1	
B	S1	S1	S1		S1(SERC)	S1(SERC)	S1(SERC)		SD2	SD2	SD2	
C	S2	S2	S2		S2(SERC)	S2(SERC)	S2(SERC)		SD3	SD3	SD3	
D	S3	S3	S3		S3(SERC)	S3(SERC)	S3(SERC)		SD4	SD4	SD4	
E									SD5	SD5	SD5	
F									SD6	SD6	SD6	
G	NEC	NEC	NEC		ERC	ERC	ERC					
H									NTC	NTC	NTC	

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

**NOTE:** The plate layout provided is a suggestion. Adjust the layout according to the number of test samples to be processed.

4. Add **20 µL** of **Working Mix** to each well according to the plate layout.
5. Add **10 µL** of DNA template to the corresponding wells. The final volume of each PCR reaction is **30 µL**.

Table 6. qPCR Reaction System

Reagents	Volume/Reaction
Working Mix	20 µL
DNA template	10 µL
<b>Total</b>	<b>30 µL</b>

6. Seal the plate with an optical film. Then, briefly centrifuge using a centrifuge rotor compatible with 96-well plates.

### Plate Document Creation and Plate Run

The following instructions apply only to the ABI 7500 instrument. For other instruments, refer to the corresponding user guide for setup instructions.

1. Create a new experiment and enter the experiment name in the **Experiment Name** field.
2. Select **Quantitation Standard Curve** mode, **TaqMan** reagents, and **Standard** mode.
3. In the **Plate Setup**, enter the **Target Name**. Select **FAM** from the **Reporter Dye** drop-down list, select **None** from the **Quencher Dye** drop-down list, and select **ROX** from 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: 300,000; 30,000; 3,000; 300; 30; 3 fg/ $\mu$ L).
5. Set up the test samples and controls according to the plate layout.
6. Set up the qPCR reaction program as specified in the following table.
7. Set the reaction volume to **30  $\mu$ L**. Click "**Start Run**" in the **Run** interface to initiate the qPCR run, and analyze the results after completion.

Table 7. qPCR Reaction Program

Step	Temperature	Cycles	Time	Signal Collection
1	60°C	1×	30 s	No
2	95°C	1×	2 min	No
3	95°C	40×	10 s	No
	64°C		30 s	<b>Yes</b>

## Results Analysis

After the qPCR run is completed, analyze the results using the general procedure. Set the threshold value to 0.2 and use **Auto Baseline** setting for the ABI7500. For other instruments, adjust the parameter setting according to the specific instrument user guide and software version. The acceptance criteria for the results are as follows:

1. The Standard curve:  $R^2 \geq 0.98$ , Eff%=90-110%.
2. The detection result for NTC should be undetermined or greater than the Ct value for SD6.
3. The Ct value for NEC should be undetermined or greater than the Ct value for SD6.
4. Ct values should be consistent across replicates, with the difference between replicates' Ct values being less than 0.5.
5. The spike recoveries for test samples and controls should be between 50% and 150%.

The formula for calculating spike recovery rate is as follows:

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

The calculation formula can be more detailed as below:

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

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**NOTE:** Calculate the concentration of the test sample (fg/ $\mu$ L) based on the standard curve. The Ct value of the test sample is valid for concentration calculation only if it falls within the assay range of the standard curve. Do not use the data to calculate the concentration if the Ct value is outside the standard curve range.

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## Typical Data

### 1. Standard Curve

Prepare a 10-fold serial dilution of HEK293T DNA Control to generate standard curve samples SD1- SD6. Perform qPCR using the resDetect™ HEK293T resDNA Ultra-Low Quantitation Kit (qPCR). The resulting standard curve should meet the following criteria: Amplification efficiency: 90-110%; Correlation coefficient ( $R^2$ ): 0.98; Slope: -3.10 to -3.80; Linear range: 300 pg/ $\mu$ L to 3 fg/ $\mu$ L.

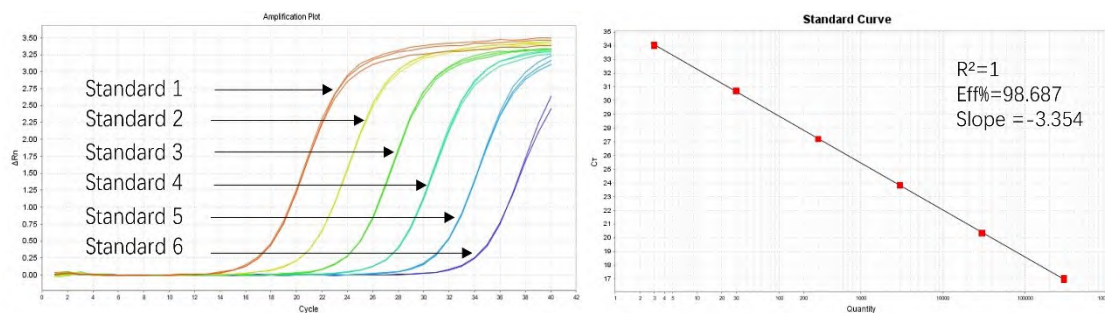


Figure 2. Standard Curve

### 2. Repeatability

Samples with concentrations of 3 pg/ $\mu$ L and 30 fg/ $\mu$ L were measured in 10 replicates. The acceptance criterion for the coefficient of variation (CV) in concentration was < 20%. The CV of detected concentrations was under 7%, demonstrating that the assay provides highly reproducible and reliable quantitation of HEK293T residual DNA.

Table 8. Repeatability Validation Results

Sample	3 pg/ $\mu$ L	30 fg/ $\mu$ L
Replicates	10	10
Detected Concentration	2.93 pg/ $\mu$ L	28.83 fg/ $\mu$ L
CV	3.17%	6.57%

### 3. Specificity Assessment

Each well of the quantitative reference standards (SD1–SD6) was spiked with 3 ng of CHO DNA, 3 ng of *E. coli* DNA or 3 ng of *Pichia* DNA. The resulting standard curves were not affected by

the presence of CHO, *E. coli* or *Pichia* DNA, demonstrating that the resDetect™ HEK293T resDNA Ultra-Low Quantitation Kit (qPCR) does not cross-react with unrelated DNA.

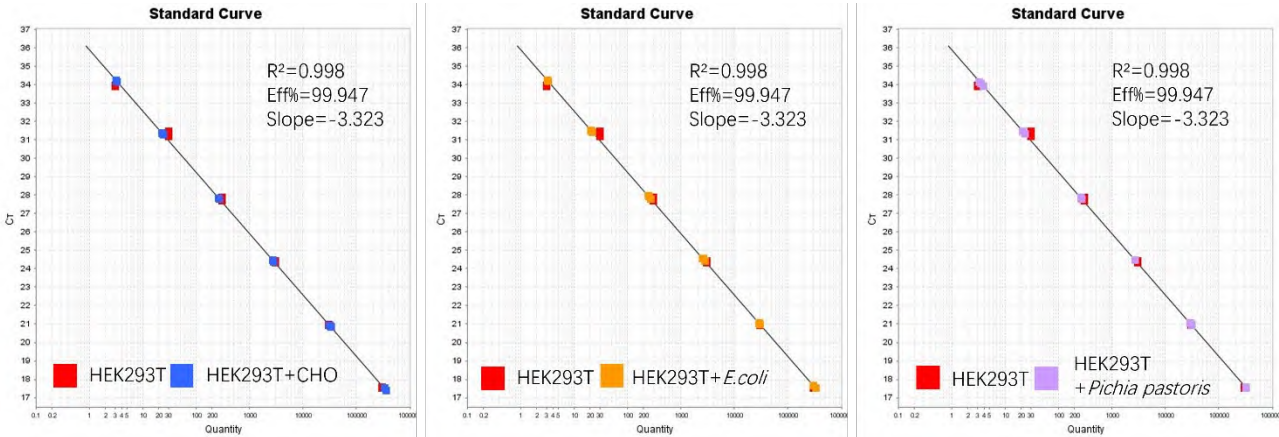


Figure 3. Specificity Validation Results

## FAQ

Table 9. Common Questions &amp; Solutions

Questions	Possible Reasons	Solutions
Poor PCR Replicate Reproducibility	<ul style="list-style-type: none"> <li>* Inaccurate pipetting</li> <li>* Insufficient sample mixing</li> </ul>	<ul style="list-style-type: none"> <li>* Check the pipettes and ensure that calibration is within the valid period before use.</li> <li>* Thoroughly mix reference standards and sample DNA for at least 20 seconds prior to dilution and pipetting to ensure sample homogeneity.</li> </ul>
Ct values of NEC < Ct values of SD6	<ul style="list-style-type: none"> <li>* NEC may become contaminated during DNA extraction or PCR setup.</li> </ul>	<ul style="list-style-type: none"> <li>* Decontaminate and clean the laboratory environment, work surfaces, and instruments before the experiment.</li> <li>* Use the Sample Dilution Buffer provided in the kit, or another suitable sample dilution buffer, as the NEC.</li> <li>* It is recommended to extract the NEC separately to check for potential contamination from the environment.</li> </ul>
Low recovery rate	<ul style="list-style-type: none"> <li>* Use of non-low-binding centrifuge tubes during extraction may reduce DNA recovery.</li> <li>* Excessive bead drying time can prevent DNA from being efficiently eluted from magnetic beads.</li> <li>* High protein content in the sample matrix may interfere with DNA extraction and quantification.</li> </ul>	<ul style="list-style-type: none"> <li>* Use low-binding centrifuge tubes during extraction to minimize DNA loss.</li> <li>* Optimize magnetic bead drying time: At high temperature, dry beads for 2 minutes; at low temperature or high humidity, dry for 3-5 minutes. During drying, observe the beads: add elution buffer when the surface appears matte. If the bead surface turns tan or yellow, the beads are over-dried, which may reduce DNA recovery.</li> <li>* For high-protein samples: consider increasing Proteinase K amount and extending digestion time to ~1 hour.</li> </ul>