



Vero residual DNA qPCR Detection Kit

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Catalog Code: VERO-DNA-PCR-100

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Product Introduction

Based on proven real-time qPCR technology, this kit is used to quantitate host - cell residual DNA from Vero cells. This kit performs rapid, specific quantitation of sub - picogram levels of residual VERO host - cell DNA. **Linearity is demonstrated by analysis of VERO cell standard DNA ranging from 3 fg/μL to 300 pg/μL.** For extraction information, refer to Residual DNA Sample Preparation Kit (magnetic bead based) (Cat. #: MB-WG-100)

Materials supplied

Component	Catalog number: VERO-DNA-PCR-100 Volume(Package size: 100 tests.)
VERO qPCR Mix	750 μL *2
VERO Primer & Probe Mix	250 μL*2
VERO DNA Standard (30ng/μL)	25 μL*2
DNA Dilution Buffer	1500 μL *4

Storage

-20°C. Suggest aliquot. Avoid repeated freeze-thaw.

Other materials required

- i. Q-PCR machine with FAM channel
Compatible with but not limited to following brand: Bio-Rad; CFX96 Optic Module; Thermo Scientific: ABI 7500; ABI Quant Studio 5; ABI Step OnePlus; LineGene 9600.
- ii. Centrifuge, vortex, pipette, laminar flow hood
- iii. Sterile low-retention centrifuge tubes. (1.5 mL or 2.0 mL)
- iv. Aerosol-resistant micropipette tips (1000 μL, 100 μL, 10 μL)
- v. PCR tubes or PCR plates.
- vi. PCR grade water.

Assay procedure

1. Preparation of the DNA Standard Dilutions for the Standard Curve

- 1.1. Thaw VERO DNA standard (30ng/μL) and Dilution buffer on ice or at 2-8°C. Gently flick and quick spin. Repeat 3 times to mix thoroughly.
- 1.2. Label ST0, ST1, ST2, ST3, ST4, ST5, ST6 on 1.5mL tubes.
- 1.3. To prepare ST0, dilute VERO DNA standard (30ng/μL) 1:10 with dilution buffer by adding 10μL VERO DNA standard to 90μL dilution buffer. (final concentration of DNA 3ng/μL) Gently flick and quick spin. Repeat 3 times to mix thoroughly.
- 1.4. Add 90μL Dilution buffer in each tube (ST1, ST2, ST3, ST4, ST5, ST6)
- 1.5. Further serial dilutions for ST1 to ST6 can be prepared starting with ST0, according to the Table 1.

Tube label	Dilution	Final concentration
ST1	10 μL ST0+90 μL DNA Dilution Buffer	300 pg/μL
ST2	10 μL ST1+90 μL DNA Dilution Buffer	30 pg/μL
ST3	10 μL ST2+90 μL DNA Dilution Buffer	3 pg/μL
ST4	10 μL ST3+90 μL DNA Dilution Buffer	300 fg/μL
ST5	10 μL ST4+90 μL DNA Dilution Buffer	30 fg/μL
ST6	10 μL ST5+90 μL DNA Dilution Buffer	3 fg/μL

Note:
The Standards ST0 to ST6 can be stored at 2~8°C for just the day of the preparation; otherwise they have to be stored at -15 to -25°C and used within one week.
When you prepare the serial dilutions of VERO DNA, follow these rules to avoid carryover contamination and to ensure proper sample preparation and quantitative PCR (qPCR) of samples:

- Use low-retention tubes.
- Label the top of each tube for identification.
- Use 2 sets of pipettes:
 - For serial dilutions and to create the standard curve
 - For sample preparation or to set up PCRs.
- Prepare the serial dilutions in an area physically separate from the test - sample preparation area.

2. Preparation of Extraction Recovery Control (ERC)

Extraction/recovery control (ERC) can be used to assess the efficiency of DNA extraction, recovery, and quantitation from test samples. Additionally, you can use ERC to verify assay and system performance. The following procedure describes the preparation of an ERC sample containing 30 pg of VERO DNA control per well for qPCR analysis.

2.1. Transfer 100µL test sample to a clean tube. (1.5 mL)

2.2. Add 10 µL ST3, mix well, label as ERC.

2.3. When you finish, extract DNA from the tubes according to the Residual DNA Sample Preparation Kit, then quantify the extracted DNA in each tube 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 assure accurate results, the amount of VERO DNA control that you add to a test sample should be approximately double 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.

3. Preparation of Negative Extraction Control (NEC)

Set up the negative extraction control as below.

3.1. Transfer 100µL sample solution into a clean 1.5 mL tube and label as NEC. For example, sample solution can be fresh medium.

3.2. Use NEC for sample preparation along with the same batch of samples to be tested. The NEC extraction is prepared.

4. Preparation of No Template Control (NTC)

4.1. Use PCR water or Dilution buffer as NTC.

5. Preparation of qPCR reaction master mix

5.1. According to the standard curve to be tested and the number of samples to be tested, calculate the number of reaction wells required, and generally make 3 replicate wells for each sample.

N= (6 Standards + 1 NTC+ 1 NEC +Test samples×2) ×3;
(Test samples×2 is because ERC is recommended to be prepared for each sample) ;
5.2. Thaw reagents on ice or 2~8 °C. Prepare qPCR reactions as described in the table below.

Item	Volume for each well
VERO qPCR mix	15 μL
VERO Primer & probe mix	5 μL
Total volume	20 μL

5.3. Calculate the total volume of qPCR reaction required for this time based on the number of wells (with 2 wells lost)

Total volume of qPCR reaction = (number of wells+2) × 20 μL

5.4. Add samples as described in the table below. Then Mix well and spin down.

Standard curve 20 μL VERO qPCR reaction master mix + 10 μL ST1/ST2/ST3/ST4/ST5/ST6	
NTC	20 μL VERO qPCR reaction master mix + 10 μL DNA Dilution buffer
NEC	20 μL VERO qPCR reaction master mix + 10 μL NEC
Test samples	20 μL VERO qPCR reaction master mix + 10 μL Test samples
ERC	20 μL VERO qPCR reaction master mix + 10 μL ERC

6. Example of layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC		S1	S1	S1	S1 ERC	S1 ERC	S1 ERC		ST6	ST6	ST6
B	NTC		S2	S2	S2	S2 ERC	S2 ERC	S2 ERC		ST5	ST5	ST5
C	NTC		S3	S3	S3	S3 ERC	S3 ERC	S3 ERC		ST4	ST4	ST4
D			S4	S4	S4	S4 ERC	S4 ERC	S4 ERC		ST3	ST3	ST3
E	NEC		S5	S5	S5	S5 ERC	S5 ERC	S5 ERC		ST2	ST2	ST2
F	NEC									ST1	ST1	ST1
G	NEC											
H												

S=Sample; NTC=No Template Control; ERC=Extraction/Recovery Control; NEC= Negative Extraction Control ST1~ST6= standard

Note.

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

7. Run the plate

7.1. Seal the plate with an optical film, then quick - spin with a centrifuge rotor that is compatible with 96 - well plates. Load the prepared 96-well plate into the qPCR machine and start the run.

8. Create the plate document, run the plate, and analyze results

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

8.1. In the Template Assay drop - down list, select Absolute Quantification.

8.2. In the Run Mode drop - down list, select Standard 7500

8.3. Entery “VERO-DNA” in the Plate name field, then click Next.

8.4. Click New Detector:

- a. Enter VERO in the Name field.
- b. Select FAM in the Report Dye drop - down list.
- c. Select (none) in the Quencher Dye drop - down list.
- d. Select a color for the detector.

8.5. Select the Instrument tab, then set thermal - cycling conditions:

- Set the thermal cycling reaction volume to 30 µL.
- For the 7500 Fast system, set the reaction to Standard.
- Set the temperature and the time as shown in the following table.

Step	Temperature	Time	Cycle	Note
1	95°C	10 minutes	1 cycle	/
2	95°C 60°C	15 seconds 1 minute	40 cycles	/ Read fluorescent signal

8.6. Save the document, then click Start to start the real - time qPCR run.

9. Analyze the results

After the qPCR run is finished, use the following general procedure to analyze the results.

9.1. In the Analysis Settings window. Manually adjust the threshold line to the appropriate position. The appropriate position is just above the highest point of the amplification curve of the negative control. For example, enter 0.2 or 0.02 in the Threshold field. Click Analyze in the tool bar, then wait while the plate is analyzed.

9.2. Select the Results tab, Standard Curve tab.

Assign values of 3000, 300, 30, 3, 0.3, and 0.03 in the Quantity column. (meaning the total amount of DNA per well. The unit is pg.)

Name them ST1, ST2, ST3, ST4, ST5, and ST6 in the corresponding Sample Name column.

9.3. Set tasks for each sample type by clicking on the Task Column drop - down list:

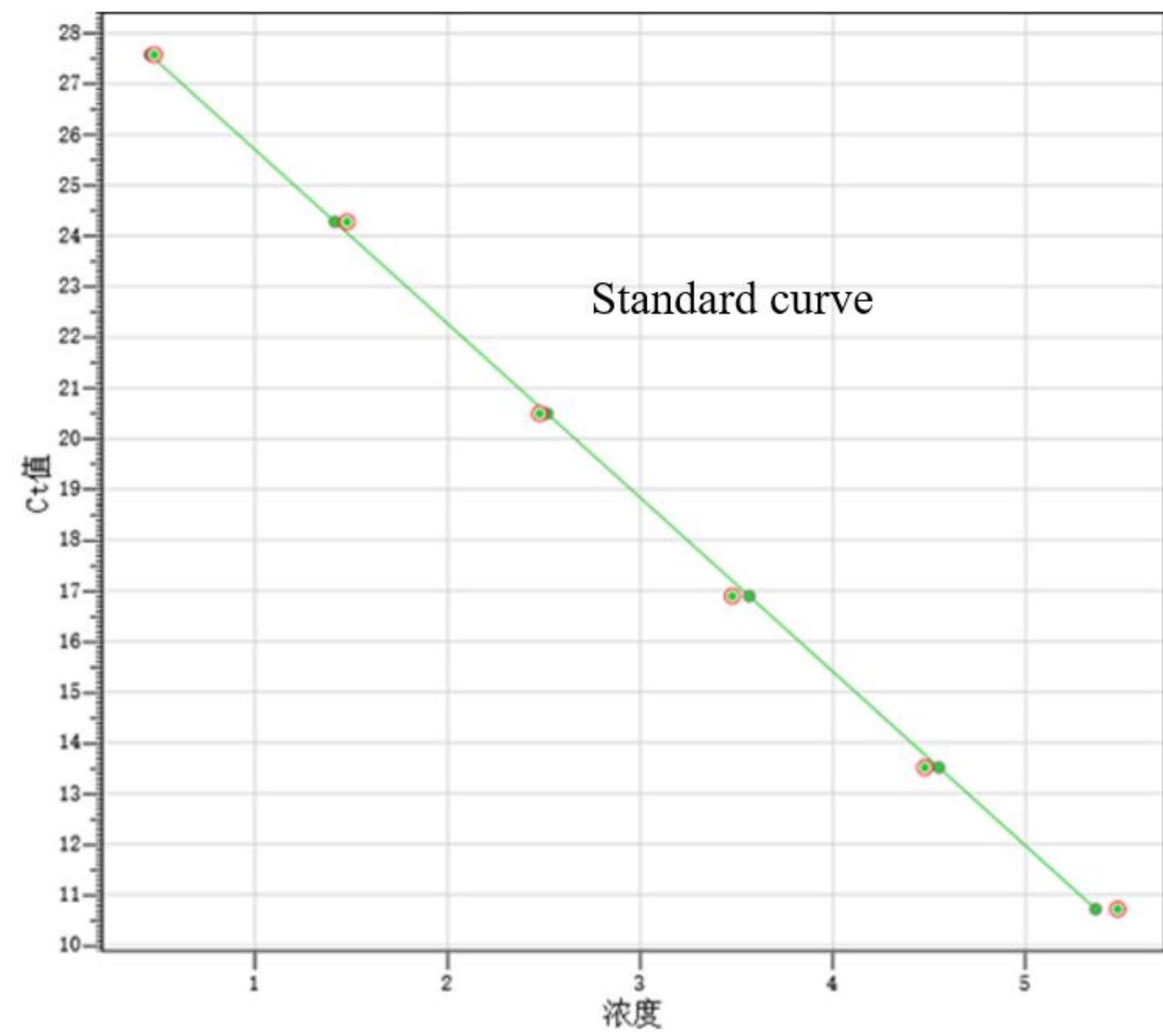
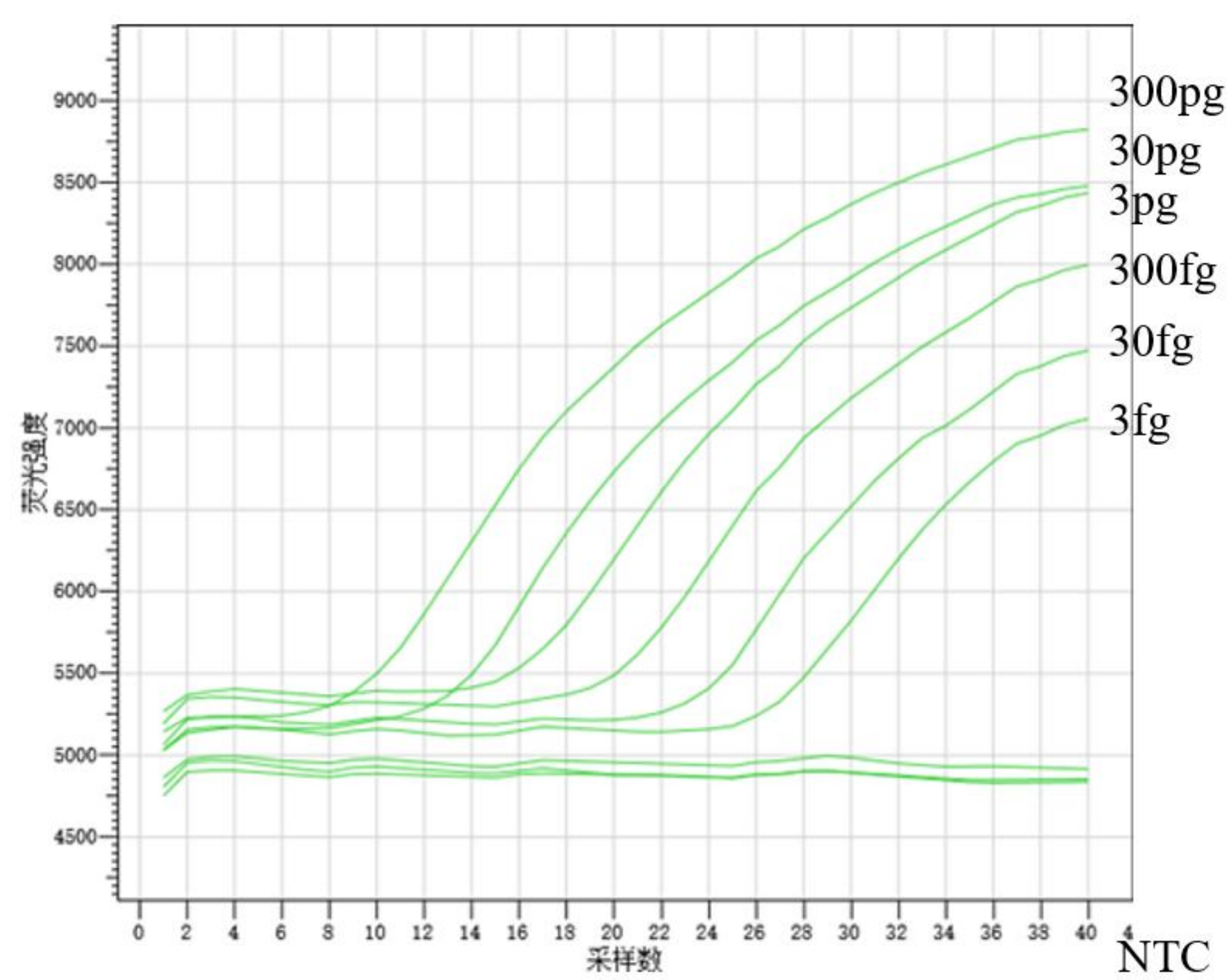
- NTC: VERO detector task = NTC
- NEC, ERC and samples wells: VERO detector task = Unknown

9.4. Select the Results tab, Standard Curve tab, then verify the Slope, Intercept, and R2 values.

9.5. Select the Report tab-Report, then review the mean quantity and standard deviation for each of the samples.

9.6. Select File-Export-Results. In the “Save as type” drop - down list, select Results Export Files (*.csv), then click Save.

10. Typical curve



Project 1: FAM channel; Intercept: 29.14; Slope: -3.44;
Deviation: 0.013; Correlation coefficient: -0.999; Efficiency: 96.4

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