



E.coli Residual DNA Detection Kit (Fluorescent LAMP)

✉ info@ezassay.com

🌐 www.ezassay.com

EZassay Biotechnology Ltd.

Catalog Code: ECO-DNA-LAM-100

CONTENTS

<u>Contents</u>	<u>Page</u>
Product Introduction	1
Materials supplied	1
Storage	1
Other materials required	1
Assay procedure	2

Product Introduction

Based on proven LAMP technology, this kit is designed for quantitative detection of residual E. coli DNA in biopharmaceuticals (Antibodies, CAR cell, Vaccines, etc.). Use the kit after extracting host - cell DNA from test samples. For achieving the better DNA recovery, it is recommended to use Residual DNA Sample Preparation Kit (magnetic bead based) (Cat. #: MB-WG-100) in combination.

This kit performs rapid, specific quantitation of sub - picogram levels of residual E.coli host - cell DNA. **Linearity is demonstrated by analysis of E.coli cell standard DNA ranging from 30 fg/ μ L to 300 pg/ μ L**, which meets the sensitivity requirements defined by WHO. For instance, parenteral administration of non-tumorigenic cell DNA should be limited to 10 ng/dose and a maximum length of 200 bp, whereas less than 100 μ g/dose of residual DNA is recommended for orally administered vaccines.

Materials supplied

Component	Catalog number: ECO-DNA-LAM-100 Volume(Package size: 100 tests.)
E coli DNA Standard (30ng/ μ L)	500 μ L
DNA Dilution Buffer	1000 μ L
E coli LAMP Buffer A	1000 μ L*2
E coli LAMP Buffer B	100 μ L

Storage

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

Other materials required

- i. Fluorescent reader with FAM channel. If Q-PCR thermal cycler is used, please make sure to turn the heat lid function off or set it to 65 °C.
- 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 μ L, 100 μ L, 10 μ L)
- 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 E.coli DNA standard (30ng/ μ L) 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 on 1.5mL tubes.
- 1.3. To prepare ST0, dilute E.coli DNA standard (30ng/ μ L) 1:10 with dilution buffer by adding 10 μ L E.coli DNA standard to 90 μ L dilution buffer. (final concentration of DNA 3ng/ μ L) Gently flick and quick spin. Repeat 3 times to mix thoroughly.
- 1.4. Add 90 μ L Dilution buffer in each tube (ST1, ST2, ST3, ST4, ST5)
- 1.5. Further serial dilutions for ST1 to ST5 can be prepared starting with ST0, according to the Table 1.

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

Note:

The Standards ST0 to ST5 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 E.coli 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 E.coli 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 E.coli DNA control added to the sample for those test samples that contain higher background levels of DNA. To assure accurate results, the amount of E.coli 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 E.coli 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 E.coli 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.

$$N = (6 \text{ Standards} + 1 \text{ NTC} + 1 \text{ NEC} + \text{Test samples} \times 2) \times 3;$$

(Test samples $\times 2$ is because ERC is recommended to be prepared for each sample) ;

5.2. Thaw reagents on ice or 2~8 °C. Prepare LAMP reactions as described in the table below.

Item	Volume for each well
E.coli LAMP Buffer A	19 μ L
E.coli LAMP Buffer B	1 μ L
Total volume	20 μ L

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

$$\text{Total volume of qPCR reaction} = (\text{number of wells} + 2) \times 20 \mu\text{L}$$

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

Standard curve	20 μ L E.coli LAMP reaction master mix + 5 μ L ST1/ST2/ST3/ST4/ST5
NTC	20 μ L E.coli LAMP reaction master mix + 5 μ L DNA Dilution buffer
NEC	20 μ L E.coli LAMP reaction master mix + 5 μ L NEC
Test samples	20 μ L E.coli LAMP reaction master mix + 5 μ L Test samples
ERC	20 μ L E.coli LAMP reaction master mix + 5 μ L ERC

Mix well and spin down.

6. Example of layout.

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

S=Sample; NTC=No Template Control; ERC=Extraction/Recovery Control; NEC= Negative Extraction Control ST1~ST5= 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 thermal cycler 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. Entry "E.coli-DNA" in the Plate name field, then click Next.

8.4. Click New Detector:

a. Enter E.coli 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 25 μ L.
- For the 7500 Fast system, set the reaction to Standard.
- Set the temperature and the time as shown in the following table.

- 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	5 minutes	1 cycle	/

8.6. Save the document, then click Start to start the real - time qPCR run. Make sure to turn off the heat lid function or set it to 65 °C.

9. Analyze the results

After the reaction 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 and 0.3 in the Quantity column. (meaning the total amount of DNA per well. The unit is pg.)

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

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

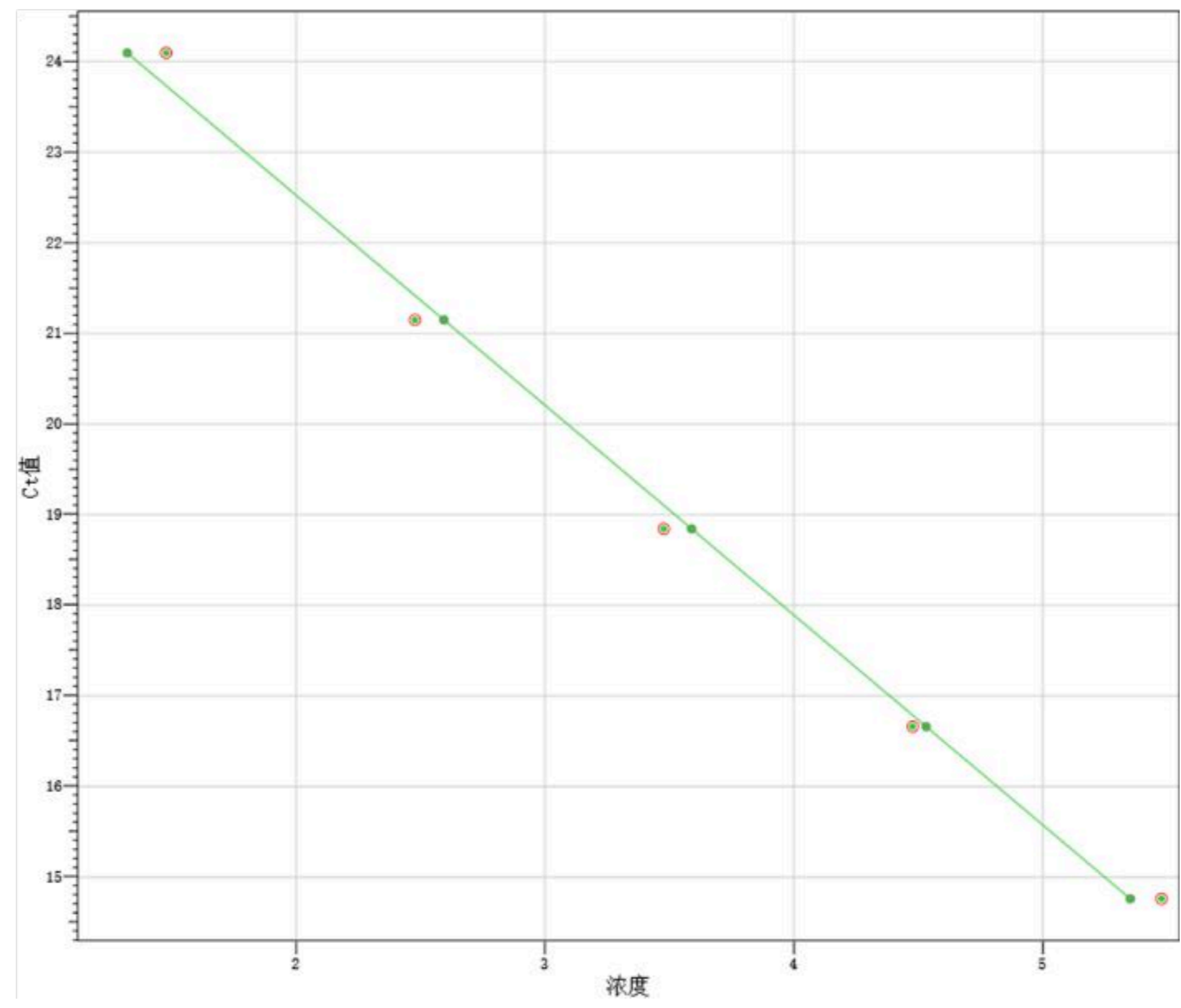
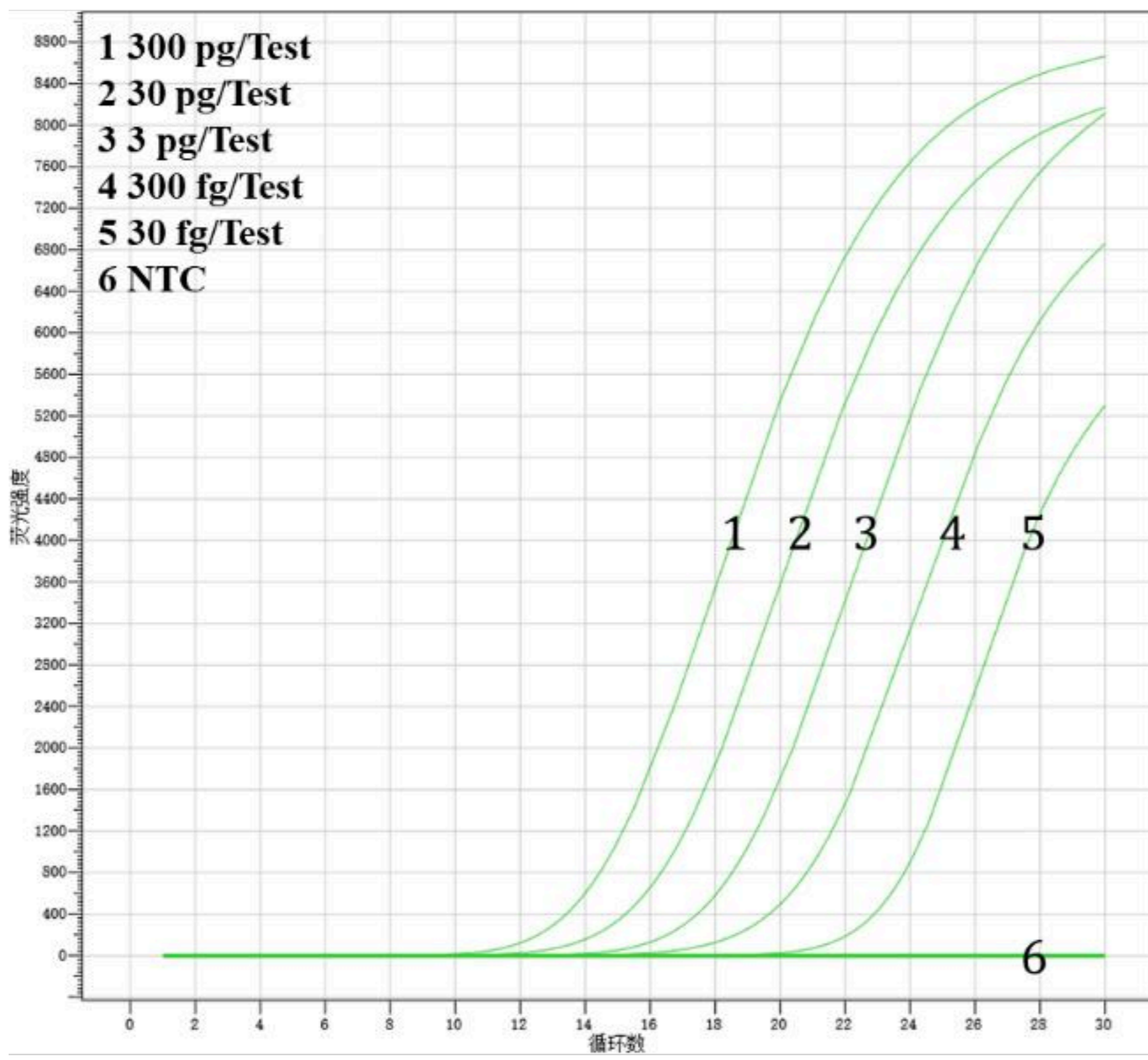
- NTC: E.coli detector task = NTC
- NEC, ERC and samples wells: E.coli detector task = Unknown

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



Professional supplier of point-of-care test products