

**SHENTEK**

# **Residual Porcine DNA Quantitation Kit User Guide**

Version: A/0

For Research Use Only

Product No.: 1101113

Reagents for 100 Reactions

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*(IMPORTANT: Please read this document carefully before experiment.)*

## 1. Product information

### ■ Product description

SHENTEK® Residual Porcine DNA Quantitation Kit is used to quantitate residual porcine DNA in biomaterials. This kit utilizes fluorescent quantitative PCR technique to perform a rapid, specific, and reliable quantitation assay at the femtogram (fg) level. For extraction information, please refer to the SHENTEK® Animal-derived Biomaterial Residual DNA Sample Preparation Kit User Guide (Product No. 1104193).

### ■ Kit contents and storage

*WARNING: Please read the Material Safety Data Sheets (MSDSs) and follow the handling instructions. Wear appropriate protective eyewear, mask, clothing, and gloves.*

Table 1. Kit components and storage

| Reagent                   | Part No. | Quantity         | Storage                      |
|---------------------------|----------|------------------|------------------------------|
| Porcine DNA Control       | NNA031   | 100 µL × 1 tube  | -20°C                        |
| AT qPCR Reaction Buffer   | NNB012   | 850 µL × 2 tubes | -20°C,<br>protect from light |
| Porcine Primer&Probe MIX  | NNC043   | 300 µL × 1 tube  |                              |
| IPC MIX                   | NNC066   | 150 µL × 1 tube  |                              |
| DNA Dilution Buffer (DDB) | NND001   | 1.5 mL × 3 tubes | -20°C                        |

The kit components can be stored at appropriate conditions for up to 24 months. Please check the expiration date on the labels.

### ■ Applied instruments, including but not limited to the following

- SHENTEK-96S Real-Time PCR System
- 7500 Real-Time PCR System

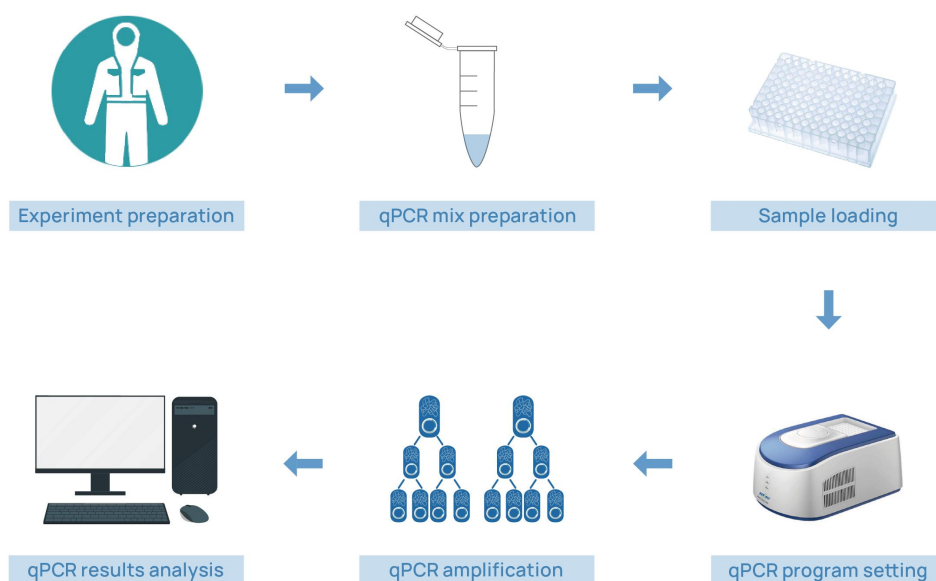
### ■ Required materials not included in the kit

- Nonstick, DNase-free & Low Retention Microfuge Tubes, 1.5 mL
- Nonstick, Low Retention Tips: 1000  $\mu$ L, 100  $\mu$ L and 10  $\mu$ L
- 96-well qPCR plates with sealing film or PCR 8-strip tubes with caps
- UNG enzyme (Please check the effective amount for best use)

## ■ Related equipment

- Real-Time PCR System
- Vortex mixer
- Benchtop microcentrifuge
- Micropipettes: 1000  $\mu$ L, 100  $\mu$ L and 10  $\mu$ L
- Microplate shaker

## ■ Workflow



## 2. Methods

### ■ Experiment preparation

1. Wear appropriate protective eyewear, mask, clothing and gloves.

2. Irradiate the tabletop, micropipettes and tubes with UV for 30 minutes, and disinfect with 75% ethanol.
3. Thaw the kit completely at 2-8°C or melt on ice, vortex and spin briefly.

### ■ DNA Control serial dilutions for the standard curve

Please check the concentration labeled on the tube containing the Porcine DNA Control prior to dilution.

1. Thaw Porcine DNA Control and DNA Dilution Buffer completely at 2-8°C or melt on ice. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times.
2. Label seven nonstick 1.5 mL microfuge tubes: ST0, ST1, ST2, ST3, ST4, ST5 and ST6.
3. Dilute the Porcine DNA Control to 30000 pg/μL with DDB in the ST0 tube. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times to mix thoroughly.
4. Add 90 μL DDB to each tube of ST1, ST2, ST3, ST4, ST5 and ST6.
5. Perform the serial dilutions according to Table 2:

Table 2. Dilution for Porcine DNA Control

| Serial dilution tube | Dilution                        | Conc. (pg/μL) |
|----------------------|---------------------------------|---------------|
| ST0                  | Dilute the DNA Control with DDB | 30000         |
| ST1                  | 10 μL ST0 + 90 μL DDB           | 3000          |
| ST2                  | 10 μL ST1 + 90 μL DDB           | 300           |
| ST3                  | 10 μL ST2 + 90 μL DDB           | 30            |
| ST4                  | 10 μL ST3 + 90 μL DDB           | 3             |
| ST5                  | 10 μL ST4 + 90 μL DDB           | 0.3           |
| ST6                  | 10 μL ST5 + 90 μL DDB           | 0.03          |

- *The remaining unused DDB need to be stored at 2-8°C. If the solution is cloudy or contains precipitates, heat at 37°C until it clear.*
- *At least five concentration of standard curve should be included. To select*

*appropriate sample dilutions, we recommend to perform method validation before sample testing.*

## ■ Sample preparation

### ➤ Extraction Reference Control (ERC) samples Preparation

According to the Porcine DNA spike concentration in ERC samples (Take the sample containing 30 ng of Porcine DNA as example), specific preparation procedure is as follows:

- (1) Take 100 µL of the test sample to a new 1.5 mL microfuge tube.
- (2) Add 10 µL of ST1 solution and mix thoroughly, label as ERC sample.

### ➤ Negative Control Sample (NCS) Preparation

Add 100 µL of DDB to a new 1.5 mL microfuge tube, and label as NCS.

NCS and samples should be prepared in same way for DNA extraction.

## ■ qPCR MIX preparation

1. Determine the number of reaction wells based on your selected standard curve, with the number of test samples and control samples. Generally, triplicates are tested for each sample.

Number of reaction wells = (6 standard points on the standard curve + 1 NTC + 1 NCS + test samples)×3

2. Prepare qPCR MIX according to the number of reaction wells in Table 3.

Table 3. qPCR MIX Preparation

| Reagents                 | Volume/reaction | Volume for 30 reaction<br>(includes 10% overage) |
|--------------------------|-----------------|--|
| AT qPCR Reaction Buffer  | 15.9 µL         | 524.7 µL   |
| Porcine primer&probe MIX | 2.8 µL          | 92.4 µL  |
| IPC MIX                  | 1.3 µL          | 42.9 µL  |
| Total volume             | 20 µL           | 660 µL   |
| UNG (optional)           | 0.1U            | 3.3 U  |

3. Mix thoroughly and place on ice, aliquot 20 µL/well into 96-well qPCR plate

or PCR 8-strip tubes.

## ■ qPCR Reaction MIX preparation

1. Prepare qPCR Reaction MIX according to Table 4, and 96-well plate layout is shown in Table 5.

Table 4. qPCR Reaction MIX Preparation

| Tubes        | Standard curve          | NTC               | NCS                        | Test sample                        |
|--------------|-------------------------|-------------------|----------------------------|------------------------------------|
| qPCR MIX     | 20 $\mu$ L              | 20 $\mu$ L        | 20 $\mu$ L                 | 20 $\mu$ L                         |
| Samples      | 10 $\mu$ L<br>ST1 - ST6 | 10 $\mu$ L<br>DDB | 10 $\mu$ L<br>purified NCS | 10 $\mu$ L purified<br>test sample |
| Total Volume | 30 $\mu$ L              | 30 $\mu$ L        | 30 $\mu$ L                 | 30 $\mu$ L                         |

Table 5. Example of 96-well Plate layout

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

- This example represents the assay for a standard curve with 6 concentration gradients (ST1-ST6), 1 NTC, 1 NCS, 5 test samples (S1-S5) and 5 ERC samples (S1 ERC-S5 ERC), with 3 replicates for each sample.
  - In specific testing, the plate layout for sample loading can be adjusted based on the sample quantity. Please refer to the example shown in Table 5.
2. Seal the 96-well plate with sealing film. Mix well in microplate shaker, then spin down the reagents for 10 seconds in centrifuge and place it on the qPCR instrument.

## ■ qPCR program setting

*NOTE: The following instructions apply only to the ABI7500 instrument with SDS v1.4. If you use a different instrument or software, refer to the applicable instrument or software documentation.*

1. Create a new document, then in the Assay drop-down list, select **Standard Curve (Absolute Quantitation)**.
2. In the Run Mode drop-down list, select **Standard 7500**, then click **Next**.
3. Click **New Detector**:
  - a. Enter **Porcine-DNA** in the Name field.
  - b. Select **FAM** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.
  - c. Select a color for the detector, then click **Create Another**.
4. Click **New Detector**:
  - a. Enter **IPC** in the Name field.
  - b. Select **VIC** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.
  - c. Select a color for the detector, then click **OK**.
  - d. Select the detectors, then click **Add** to add the detectors to the document.
5. Select **ROX** as the passive reference dye, then Click **Next**.
6. Select the applicable set of wells for the samples, then select **Porcine-DNA** detector and **IPC** detector for each well.
7. Select **Finish**, and then set thermal-cycling conditions:
  - a. Set the thermal cycling reaction volume to 30  $\mu$ L.
  - b. Set the temperature and time as follow in Table 6:

Table 6. qPCR running temperature and time

| Step                          | Temp. | Time(mm:sec) | Cycles |
|-------------------------------|-------|--------------|--------|
| UNG treatment #<br>(optional) | 25°C  | 10:00        | 1      |
| Activation                    | 95°C  | 10:00        | 1      |
| Denaturation                  | 95°C  | 00:15        | 40     |
| Annealing/extension           | 60°C* | 00:40        |        |

#UNG treatment step is needed if UNG enzyme is included in qPCR reaction.

\*Instrument will read the fluorescence signal during this step.

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

## ■ Results analysis

1. Select **Set up** tab, then set tasks for each sample type by clicking on the Task Column drop-down list:

- a. NTC: target DNA detector task = **NTC**
- b. NCS, test samples, and ERC wells: target DNA detector task = **Unknown**

2. Set up the standard curve as shown in table 7:

Table 7. Settings for Standard curve


| Tube label | Task     | Quantity (pg/μL) |
|------------|----------|------------------|
| ST1        | Standard | 3000             |
| ST2        | Standard | 300              |
| ST3        | Standard | 30               |
| ST4        | Standard | 3                |
| ST5        | Standard | 0.3              |
| ST6        | Standard | 0.03             |

3. Select the **Results** tab, then select Amplification Plot.

4. In the Data drop-down list, select **Delta Rn vs Cycle**.

5. In the Analysis Settings window, enter the following settings:

- a. Select **Manual Ct**.
- b. In the Threshold field, Porcine-DNA enter 0.02 and IPC enter 0.02.

- c. Select **Automatic Baseline**.
6. Click the button  in the toolbar, then wait the plate analyzing.
7. Select the **Result** tab > > **Standard curve** tab, then verify the Slope, Intercept and R<sup>2</sup> values.
8. Select the Report tab, then achieve the mean quantity and standard deviation for each sample.
9. Select **File > > Export > > Results**. In the Save as type drop-down list, select **Results Export Files**, then click **Save**.
10. In the Report panel of Results, the 'Mean Quantity' column shows the detection values of NTC, NCS, test sample, and ERC sample, in pg/μL.

*Note: The parameter settings of the result analysis should be configured on the specific model and the software version, and generally can also be automatically interpreted by the instrument.*

11. To analyze the Ct value of IPC, normally the mean Ct-IPC value of the sample should be within  $\pm 1.0$  of the NCS Ct-IPC value. If the mean Ct-IPC value of the sample is significantly higher than the Ct-IPC value of the NCS, this indicates that the sample may be inhibitory to the assay. It is recommended testing the ERC samples at the same time, and take the sample recovery rate result as the criterion.
12. The recovery rate of ERC samples is calculated based on the value of test samples and the ERC samples. The recovery rates should be between 50% and 150%.
13. The Ct value of NCS should be larger than the mean Ct value of the lowest concentration in the standard curve. If the validated limit of quantitation (LOQ) concentration is less than the lowest concentration in the standard curve, the value of the NCS should be less than the concentration of LOQ.
14. The results of NTC should be 2 larger than the mean Ct value of the lowest concentration in the standard curve, or specific criteria should be set according

to the laboratory's verification results.

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## Support & Contact

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