NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

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SHENTEK

Residual Bovine DNA Quantitation Kit
User Guide

Version: A/0
For Research Use Only
Product No.: 1101112
Reagents for 100 Reactions

Biofargo, Inc.

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

1. Product information

■ Product description

SHENTEK® Residual Bovine DNA Quantitation Kit is used to quantitate residual bovine 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
Bovine DNA Control	NNA032	100 μL × 1 tube	-20℃
Bovine Primer&Probe MIX	NNC042	300 μL × 1 tube	-20℃, protect from light
AT qPCR Reaction Buffer	NNB012	850 μL × 2 tubes	
IPC MIX	NNC066	150 μL × 1 tube	
DNA Dilution Buffer (DDB)	NND001	1.5 mL × 3 tubes	-20℃

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

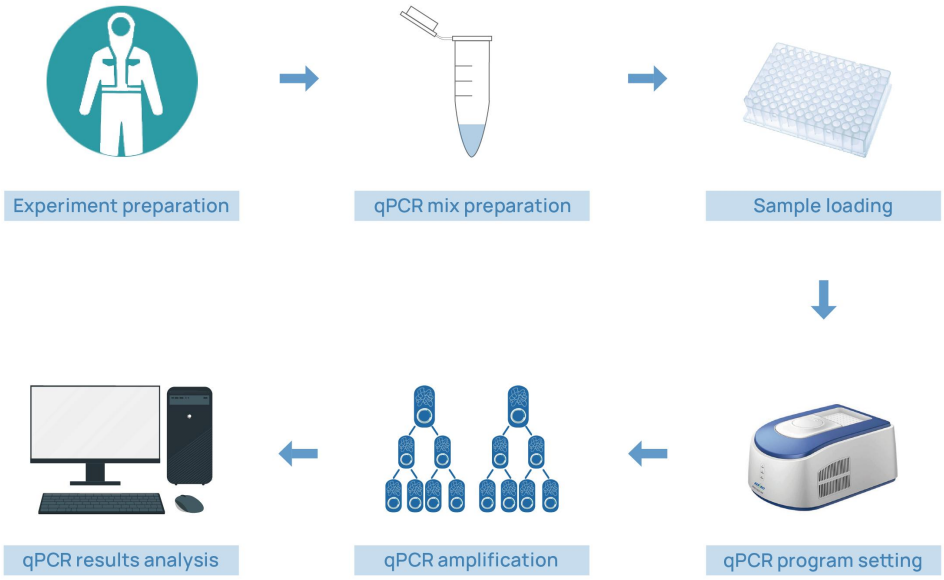
■ Required materials not included in the kit

- Nonstick, DNase-free & Low Retention Microfuge Tubes, 1.5 mL
- Nonstick, Low Retention Tips: 1000 µL, 100 µL and 10 µL
- 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 µL, 100 µL and 10 µL
- 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 Bovine DNA Control prior to dilution.

1. Thaw Bovine 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 Bovine 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 Bovine 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℃. If the solution is cloudy or contains precipitates, heat at 37℃ 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 Bovine DNA spike concentration in ERC samples (Take the sample containing 30 ng of Bovine 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 (includes 10% overage)**
AT qPCR Reaction Buffer	15.9 μL	524.7 μL
Bovine primer&probe MIX	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL
UNG enzyme (optional)	0.1 U	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 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST6	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 5. Example of 96-well Plate layout

NTC		S1	S1	S1	S1 ERC	S1 ERC	S1 ERC		ST6	ST6	ST6	A
NTC		S2	S2	S2	S2 ERC	S2 ERC	S2 ERC		ST6	ST6	ST6	B
NTC		S3	S3	S3	S3 ERC	S3 ERC	S3 ERC		ST5	ST5	ST5	C
		S4	S4	S4	S4 ERC	S4 ERC	S4 ERC		ST4	ST4	ST4	D
NCS		S5	S5	S5	S5 ERC	S5 ERC	S5 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 Bovine -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 Bovine-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 μL.
   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# (optional)	25℃	10:00	1
Activation	95℃	10:00	1
Denaturation	95℃	00:15	40
Annealing/extension	63℃*	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, Bovine-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^2 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 ±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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