



User Manual

Disclaimer: Products are intended for research use only

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Residual MDCK DNA Size Analysis Kit

User Guide

Version: A/0
For Research Use Only
Product No.: 1103175
Reagents for 4×100 Reactions

Biofargo, Inc.

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

1. Product information

■ Product description

SHENTEK® Residual MDCK DNA Size Analysis Kit is used to quantitate fragment sizes of residual MDCK DNA in different stages of biopharmaceutical products, from in-process samples to final products.

This kit utilizes real-time PCR technique to perform rapid and specific quantitation of residual MDCK DNA fragments (FAM) in samples. It is designed to amplify four different fragments (84bp, 142bp, 204bp and 504bp) for the accurate determination of their size distribution at LOD of femtograms (fg). For extraction information, please refer to the SHENTEK® Residual Host Cell DNA Sample Preparation Kit User Guide (Product No. 1104191).

■ 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 |
|---------------------------|----------|------------------|---------|
| MDCK DNA Control | NNA006 | 50 µL × 1 tube | -20°C |
| qPCR Reaction Buffer | NNB001 | 850 µL × 8 tubes | |
| MDCK Primer&Probe MIX-84 | NNC025 | 300 µL × 1 tube | |
| MDCK Primer&Probe MIX-142 | NNC026 | 300 µL × 1 tube | |
| MDCK Primer&Probe MIX-204 | NNC027 | 300 µL × 1 tube | |
| MDCK Primer&Probe MIX-504 | NNC028 | 300 µL × 1 tube | |
| IPC MIX | NNC070 | 550 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

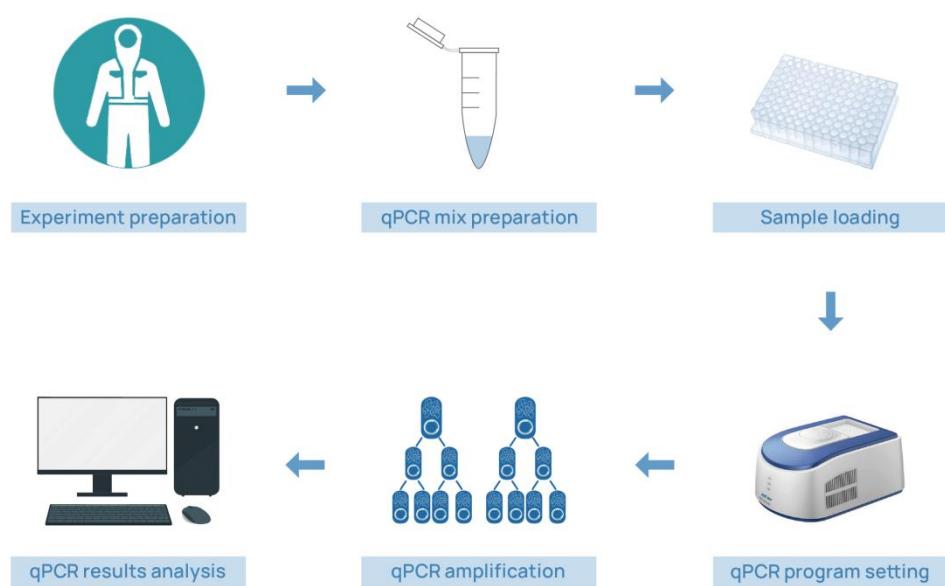
- SHENTEK-96S Real-Time PCR System
- ABI 7500 Real-Time PCR System
- LightCycler 480II Real-Time PCR System

■ Required materials not included in the kit

- Low retention, RNase/DNase-free, sterile microcentrifuge tubes
- PCR 8-well strip tubes with caps or 96-well plate with seals
- Low retention filter tips: 1000 μ L, 100 μ L and 10 μ L

■ Related equipments

- Real-Time PCR System
- Benchtop microcentrifuge
- Vortex mixer
- Pipettes: 1000 μ L, 100 μ L and 10 μ L
- Microplate and microtube shaker

■ Workflow

2. Methods

■ Experiment preparation

1. Wear appropriate protective eyewear, mask, clothing and gloves.
2. Irradiate the tabletop, pipettes 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

Note: The kit contains four MDCK primer & probe mixes for different fragment lengths. Please set up four separate standard curves corresponding to each fragment length.

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

Prepare four sets of MDCK DNA Control solution with DNA Dilution Buffer (DDB) following the serial dilution procedure below:

1. Thaw MDCK DNA Control and DDB 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 1.5 mL microcentrifuge tubes: ST0, ST1, ST2, ST3, ST4, ST5 and ST6.
3. Dilute the DNA Control to 3000 pg/μL with DDB in the ST0 tube. Calculate the volume of DDB to prepare the ST0:

$$\frac{\text{DNA Control conc. (A)} \times 1000 \text{ pg/ng} \times \text{Volume of DNA Control (B)}}{3000 \text{ pg/uL}} - \text{Volume of DNA Control (B)}$$

For example:

The concentration on the label of the DNA Control is 30.9 ng/μL (A), pipette 10 μL (B) of the DNA Control to the ST0 tube. Add the below volume to reach 3000 pg/uL.

$$\frac{30.9 \text{ ng/uL} \times 1000 \text{ pg/ng} \times 10 \text{ uL}}{3000 \text{ pg/uL}} - 10 \text{ uL} = 93 \text{ uL}$$

4. Vortex to mix well and quickly spin down the ST0 tube for 3-5 seconds in microcentrifuge, and repeat 3 times to mix thoroughly.
5. Add 180 μ L DDB to each tube of ST1, ST2, ST3, ST4, ST5 and ST6.
6. Perform the serial dilutions according to Table 2:

Table 2. Dilution for MDCK DNA Control

| Serial dilution tube | Dilution | Conc. (pg/ μ L) |
|----------------------|----------------------------------|---------------------|
| ST0 | Dilute the DNA Control with DDB | 3000 |
| ST1 | 20 μ L ST0 + 180 μ L DDB | 300 |
| ST2 | 20 μ L ST1 + 180 μ L DDB | 30 |
| ST3 | 20 μ L ST2 + 180 μ L DDB | 3 |
| ST4 | 20 μ L ST3 + 180 μ L DDB | 0.3 |
| ST5 | 20 μ L ST4 + 180 μ L DDB | 0.03 |
| ST6 | 20 μ L ST5 + 180 μ L DDB | 0.003 |

- *The remaining unused DDB needs 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 concentrations of standard curve should be included. To select appropriate sample dilutions, we recommend performing method validation before sample testing.*

■ Sample preparation

- Negative Control Sample (NCS) Preparation

Add 100 μ L of DDB to a new 1.5 mL microcentrifuge tube, and label as NCS.

NCS should be processed in the same procedures as test sample preparation before testing.

■ qPCR MIX preparation

1. Determine the number of reaction wells based on the 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) \times 3

2. Prepare qPCR MIX according to the number of reaction wells in Table 3-6.

Table 3. qPCR MIX-84 preparation

| Reagents | Volume/reaction | Volume for 30 reaction (includes 10% overage) |
|--------------------------|-----------------|--|
| qPCR Reaction Buffer | 15.9 μ L | 524.7 μ L |
| MDCK Primer&Probe MIX-84 | 2.8 μ L | 92.4 μ L |
| IPC MIX | 1.3 μ L | 42.9 μ L |
| Total volume | 20 μ L | 660 μ L |

Table 4. qPCR MIX-142 preparation

| Reagents | Volume/reaction | Volume for 30 reaction (includes 10% overage) |
|---------------------------|-----------------|--|
| qPCR Reaction Buffer | 15.9 μ L | 524.7 μ L |
| MDCK Primer&Probe MIX-142 | 2.8 μ L | 92.4 μ L |
| IPC MIX | 1.3 μ L | 42.9 μ L |
| Total volume | 20 μ L | 660 μ L |

Table 5. qPCR MIX-204 preparation

| Reagents | Volume/reaction | Volume for 30 reaction (includes 10% overage) |
|---------------------------|-----------------|--|
| qPCR Reaction Buffer | 15.9 μ L | 524.7 μ L |
| MDCK Primer&Probe MIX-204 | 2.8 μ L | 92.4 μ L |
| IPC MIX | 1.3 μ L | 42.9 μ L |
| Total volume | 20 μ L | 660 μ L |

Table 6. qPCR MIX-504 preparation

| Reagents | Volume/reaction | Volume for 30 reaction (includes 10% overage) |
|---------------------------|-----------------|--|
| qPCR Reaction Buffer | 15.9 μ L | 524.7 μ L |
| MDCK Primer&Probe MIX-504 | 2.8 μ L | 92.4 μ L |
| IPC MIX | 1.3 μ L | 42.9 μ L |
| Total volume | 20 μ L | 660 μ L |

For simultaneous detection of the four fragments, please prepare at least 120 μ L

template DNA for four assays.

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 7-10, and a 96-well plate layout template is shown in Table 11.

Table 7. qPCR Reaction MIX-84 preparation

| Tubes | Standard curve | NTC | NCS | Test sample |
|--------------|--------------------|-----------|-----------------------|-------------------------------|
| qPCR MIX-84 | 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 8. qPCR Reaction MIX-142 preparation

| Tubes | Standard curve | NTC | NCS | Test sample |
|--------------|--------------------|-----------|-----------------------|-------------------------------|
| qPCR MIX-142 | 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 9. qPCR Reaction MIX-204 preparation

| Tubes | Standard curve | NTC | NCS | Test sample |
|--------------|--------------------|-----------|-----------------------|-------------------------------|
| qPCR MIX-204 | 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 10. qPCR Reaction MIX-504 Preparation

| Tubes | Standard curve | NTC | NCS | Test sample |
|--------------|--------------------|-----------|-----------------------|-------------------------------|
| qPCR MIX-504 | 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 11. Example of 96-well plate layout

Plate 1:

| MIX-84 | | | | | | MIX-142 | | | | | | A |
|--------|-----|-----|-----|-----|-----|---------|-----|-----|-----|-----|-----|---|
| NTC | NTC | NTC | | | | NTC | NTC | NTC | | | | |
| NCS | NCS | NCS | | | | NCS | NCS | NCS | | | | B |
| | | | ST6 | ST6 | ST6 | | | | ST6 | ST6 | ST6 | C |
| | | | ST5 | ST5 | ST5 | | | | ST5 | ST5 | ST5 | D |
| S1 | S1 | S1 | ST4 | ST4 | ST4 | S1 | S1 | S1 | ST4 | ST4 | ST4 | E |
| S2 | S2 | S2 | ST3 | ST3 | ST3 | S2 | S2 | S2 | ST3 | ST3 | ST3 | F |
| S3 | S3 | S3 | ST2 | ST2 | ST2 | S3 | S3 | S3 | ST2 | ST2 | ST2 | G |
| S4 | S4 | S4 | ST1 | ST1 | ST1 | S4 | S4 | S4 | ST1 | ST1 | ST1 | H |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |

Plate 2:

| MIX-204 | | | | | | MIX-504 | | | | | | A |
|---------|-----|-----|-----|-----|-----|---------|-----|-----|-----|-----|-----|---|
| NTC | NTC | NTC | | | | NTC | NTC | NTC | | | | |
| NCS | NCS | NCS | | | | NCS | NCS | NCS | | | | B |
| | | | ST6 | ST6 | ST6 | | | | ST6 | ST6 | ST6 | C |
| | | | ST5 | ST5 | ST5 | | | | ST5 | ST5 | ST5 | D |
| S1 | S1 | S1 | ST4 | ST4 | ST4 | S1 | S1 | S1 | ST4 | ST4 | ST4 | E |
| S2 | S2 | S2 | ST3 | ST3 | ST3 | S2 | S2 | S2 | ST3 | ST3 | ST3 | F |
| S3 | S3 | S3 | ST2 | ST2 | ST2 | S3 | S3 | S3 | ST2 | ST2 | ST2 | G |
| S4 | S4 | S4 | ST1 | ST1 | ST1 | S4 | S4 | S4 | ST1 | ST1 | ST1 | H |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |

- This example represents four assays, including selected standard curve points of MDCK DNA Control (ST1-ST6), 1 NTC, 1 NCS, 4 test samples (S1-S4), with 3 replicates for each sample.
- The plate layout for sample loading can be adjusted based on the sample quantity.

2. Seal the 96-well plate with sealing film. Mix well in microplate shaker, then spin down the reagents for 10 seconds in microcentrifuge 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. Click **New Detector**, then enter MDCK-84 in the Name field, select **FAM** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.
3. Create new detector for MDCK-142, MDCK-204 and MDCK-504, separately as step2.
4. Click **New Detector**, then enter IPC in the Name field. Select **VIC** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.
5. Select **ROX** as the passive reference dye, then click **Next**.
6. Select the applicable set of wells for the samples, then select the corresponding detector for each well.
7. Select **Finish**, and then set thermal-cycling conditions:
 - a. Set the thermal cycling reaction volume to 30 μ L.
 - b. Set the temperature and time as follows in Table 12:

Table 12. qPCR running temperature and time

| Step | Temp. | Time(mm:sec) | Cycles |
|--------------|-------|--------------|--------|
| Activation | 95°C | 10:00 | 1 |
| Denaturation | 95°C | 00:15 | 40 |
| Annealing | 60°C | 00:30 | |
| Extension | 72°C* | 01:30 | |

*Instrument will read the fluorescence signal during this step.

8. Save the document, then click **Start** to start the 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 = **Unknown**
2. Set up the standard curve as shown in table 13:

Table 13. Settings for Standard curve

| Tube label | Task | Quantity (pg/µL) |
|------------|----------|------------------|
| ST1 | Standard | 300 |
| ST2 | Standard | 30 |
| ST3 | Standard | 3 |
| ST4 | Standard | 0.3 |
| ST5 | Standard | 0.03 |
| ST6 | Standard | 0.003 |

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, 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 R² 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**.

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

10. Set the value of DNA size-84 to be 100%, calculate the percentage of the DNA size of 142, 204 and 504.
11. 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. We recommend to test the ERC samples at the same assay, and take the sample recovery rate as the criterion.
12. The Ct value of NCS should be larger than the mean Ct value of the lowest concentration in the standard curve, and shows normal amplification curve in the VIC signal channel.
13. The Ct value of NTC should not be less than 35.00 cycles or undetermined, meanwhile shows normal amplification curve in the VIC signal channel.

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Support & Contact

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