DUCKY BIO

User Guide

resDNASEQ CHO
Residual DNA Quantitation kit

Cat. No. D24011202 **100 reactions of 20 µL final volume each**

Store the kit at -15 to -25°C.

Version 01

Ducky Biotechnology Co., Ltd.

This product is for research purposes only. Not for use in diagnostic procedures.

The information in this guide is subject to change without notice.

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Content

CHAPTER 1 Product description

1.1 Application

The resDNASEQ CHO Residual DNA Quantitation kit is designed for the quantification of residual DNA from CHO, in cell lines which are used for production of biopharmaceutical products. Use the kit after you extract host cell DNA from test samples. For extraction information, see the Residual DNA Sample Preparation Kits User Guide (Cat. No. S24080201).
To generate the standard curve used to quantitate the DNA in test samples, the CHO assays require five dilutions. DNA control from CHO cells (CHO DNA Control) is included in the kit. In addition, the kits use an internal positive control (IPC) to evaluate the performance of each PCR reaction. UDG enzyme is used in this kit to avoid the contamination of amplification products.

1.2 Specificity and Sensitivity

The resDNASEQ CHO Residual DNA Quantitation kit use TaqMan™ quantitative PCR to perform rapid, specifc quantitation of femtogram levels of residual host-cell or plasmid DNA. The assay is accurate and reliable across a broad range of sample types, from in-process samples to final product.

The kit was developed to meet the sensitivity requirements defined by WHO (10 ng CHO DNA per therapeutic dose). Linearity is demonstrated by analysis of standard DNA from CHO ranging from 5 pg/mL to 30 μg/mL.

Limit of Detection (LOD): 0.01 pg/mL
Limit of Quantification (LOQ): 0.3 pg/mL

CHAPTER 2 The Reagents and Samples

2.1 Required materials supplied

Table 1 resDNASEQ CHO Residual DNA Quantitation kit (Cat. No. D24011202)

Contents	Cap color	Amount	Storage
CHO Real-Time PCR Reagents			
CHO DNA qPCR MIX		2×1.0 mL	−15°C to −25°C before first use, protect from light; 2−8°C for 1 week after first use.
PCR Negative Control		1×1.0 mL	−15°C to −25°C
CHO Internal Positive Control		1.0 mL	
CHO DNA Control, 30 ng/μL		40 μL	
Dilution Buffer		7 mL	

Number of Tests: The kit contains sufficient reagents to run 100 PCR reactions each with a final reaction volume of 20 μL.

◆ This product is shipped on dry ice.
◆ This kit is stable until the expiration date printed on the label when stored at −15°C to −25°C.
◆ This kit is stable at +2 to +8°C for 1 week.

Assay Time: Total time-to-result (without sample preparation): approximately **1.5 hours;**
Total time-to-result (with sample preparation): approximately **2 hours.**

2.2 Additional Equipment and Reagents Required

application	Item
Miscellaneous	Standard laboratory equipment
	Disposable gloves
	Pipettes
	Nuclease-free, DNA-free aerosol-resistant pipette tips
	Nonstick, Nuclease-free, Microfuge Tubes to prepare working solution, dilutions.
	To minimize risk of nuclease contamination, autoclave all vessels and use alcohol wipes

For nucleic acid isolation (manual sample preparation)	Residual DNA Sample Preparation Kit(Cat. No. Cat. No. S24020201)
For the PCR workflow	Laminar flow hood
	Real-time PCR instrument for detection in FAM &ROX channel including accessories and consumables. We recommend the SLAN 96p, ABI 7500.
	Multiwell plates: plates are adapted to your PCR instrument.
	Centrifuge is adapted to your perforated plates.

CHAPTER 3 Before You Begin

3.1 Precautions

To avoid contamination, perform the workflow setup under DNA-free conditions. This includes:

- Prepare and pipette all solutions with nuclease-free, DNA-free equipment and consumables.
- UV-treat or the laminar flow hood prior to pipetting.
- Use sterile single-use gloves and freshly laundered laboratory coats.
- Close tubes immediately after pipetting.
- Spatial segregation of the sequential workflow steps.

Rooms	Workflow Step
Sample preparation room	Extraction and purification of test samples,including preparation of recovery control sample.
Master mix preparation room	Master mix preparation and pipetting of PCR Negative Control to the NTC wells.
PCR room for setup and amplification run	Dilution and pipetting of samples and PCR Positive Control to the PCR plate. Running the PCR Instrument.

CHAPTER 4 Manual Sample Preparation

In combination with this kit, a manual sample preparation with the Residual DNA Sample Preparation Kit (Cat. No. S24020201) is recommended.

Note that due to different types of matrices (i.e. high protein amounts or very high DNA amount), the test samples should be appropriately diluted before running the sample preparation. For this purpose, use the Dilution Buffer provided in this kit (Nature Vial).

The correct pH of the sample solutions (pH 7.5) is very important for good DNA recovery.

4.1 Procedure for Preparing DNA from 100 µL Test Sample

Figure 1 Describes the protocol for preparing DNA from the 100µl test sample. Use Workflow B for each test sample to be analyzed.

Run once per sample preparation:

- Workflow A: to prepare the Workflow Negative Control.
- Workflow B: to prepare the Workflow Test Sample.
- Workflow C: to prepare the Workflow Positive Control.
- Optional: Workflow D to prepare the Recovery Control.

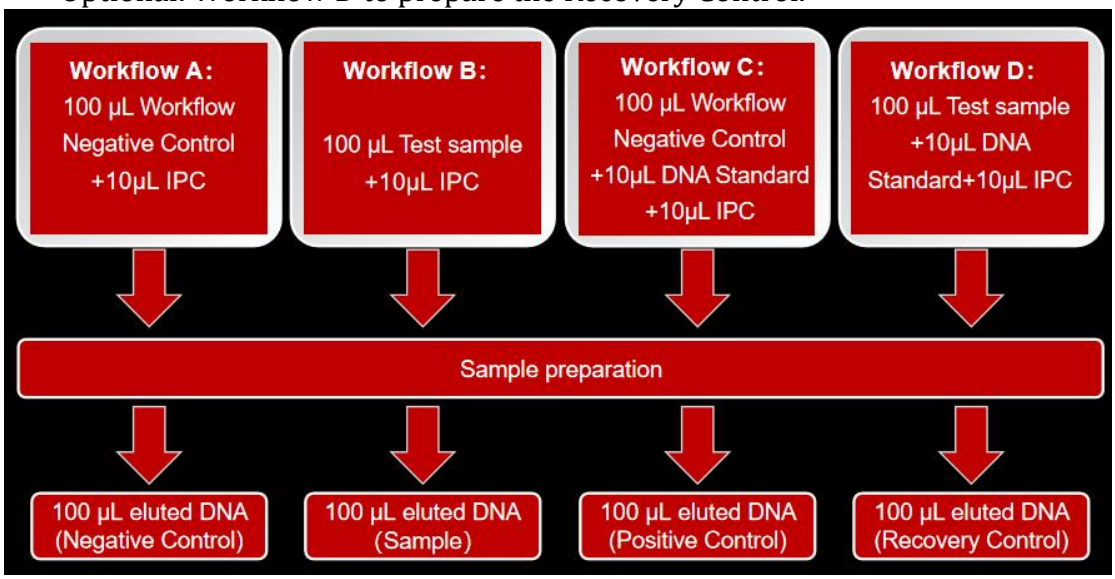


Figure 1 Experimental overview of the Sample preparation.

For the recovery control, use a DNA concentration which is 5 to 10 times higher than in the test sample. The spike of a DNA Standard depends on the concentration of residual DNA in the test samples: For low or very low DNA concentration, use the SD 3; for high DNA concentration, use SD1 or SD2 (for SD1, SD2, and SD3, see CHAPTER 5.1.2).

IPC Sample (Internal Positive Control) is offered in the resDNASEQ E.coli Residual DNA Quantitation kit.

Instead of the Workflow Negative Control you can also use the Dilution Buffer.

$$Recovery = \frac{The\ concentration\ of\ a\ sample\ with\ added\ DNA(pg/\mu L)*Elution\ volume(100\mu L) - Sample\ concentration(pg/\mu L)*Elution\ volume(100\mu L)}{The\ amount\ of\ DNA\ added/pg} \times 100\%$$

CHAPTER 5 Setting Up the PCR Experiment

5.1 Prepare the control DNA serial dilutions for the standard curve

5.1.1 Guidelines for standard dilutions

- Prepare the serial dilutions in an area physically separate from the sample preparation area.
- Use pipettors for the serial dilutions that are different from those used for sample preparation or PCR setup.
- Use nuclease-free, DNA-free tubes.
- Briefly centrifuge to collect all the liquid at the bottom before making the next dilution.
- Vortex each tube to mix the contents **thoroughly** before each dilution step.

5.1.2 Prepare the control serial dilutions.

1. Label nonstick 1.5-mL microfuge tubes: NTC, SD1, SD2, SD3, SD4, SD5, SD6.
2. Add 50 µL of DNA Dilution Buffer to tube NTC. Put aside.
3. Add 990 µL of DNA Dilution Buffer to tube SD1.
4. Add 450 µL of DNA Dilution Buffer to tubes SD2, SD3, SD4, SD5, SD6.
5. Remove the tube of DNA control (30 ng/µL) from the freezer.
6. After the DNA thaws, vortex it gently for 30 seconds, then briefly centrifuge.
7. Perform the serial dilutions:

- Add 10 µL of the DNA control to the tube that is labeled SD1, then vortex thoroughly and briefly centrifuge.
- Transfer 50 µL of the DNA from tube SD1 to tube SD2, then vortex thoroughly and briefly centrifuge.
- Continue to transfer 50 µL of DNA from the previous dilution tube to the next dilution tube until you add DNA to tube SD6. Final dilutions are shown in the table. After each transfer, vortex thoroughly, then centrifuge briefly.

Serial dilution (SD) tube	Dilution	pg DNA/µL (20 µL of the diluted DNA used in final 40 µL of PCR reaction)
Control	DNA control tube	300,00 pg/µL (30ng/µL)
SD 1	10 µL DNA control + 990 µL DDB	3,00 pg/µL
SD 2	50 µL SD1 + 450 µL DDB	30 pg/µL
SD 3	50 µL SD2 + 450 µL DDB	3 pg/µL
SD 4	50 µL SD3 + 450 µL DDB	0.3 pg/µL
SD 5	50 µL SD4 + 450 µL DDB	0.03pg/µL
SD 6	50 µL SD5 + 450 µL DDB	0.003pg/µL

8. Store the DNA dilution tubes:

Temprature	For use

4°C	Same day
–20°C	≤1 week
–20°C	SD1 in single-use aliquots ≤6 months

5.2 Preparation of the PCR Plate and PCR Run

5.2.1 Prepare the PCR plate

1. Perform laminar flow hood cleaning (using bleach, then ethanol or other disinfectant reagents) in the PCR room.
2. Wipe the pipetting tools with 10% bleach followed by 70% ethanol or other disinfectants. After that, the air in the laboratory should be changed before performing PCR experiments.
3. Determine the number of reactions needed for the controls and test samples that you will quantify.
4. Thaw all kit reagents completely at room temperature, votex them throughly, and briefly centrifuge.
5. Add 20 µL PCR reaction mix to each well.
6. Add 20 µL of PCR NTC to the appropriate wells.
7. Add 20 µL of standard dilutions to the appropriate wells.

Note: Use different sets of pipettors to dispense test sample and standard curve dilutions to avoid cross-contamination of test samples.

8. Add 20 µL of the sample material (Positive Control, Sample, Recovery Control, Negative Control) to the corresponding well prefilled with 20 µL CHO DNA qPCR MIX. Adding samples to the wells are shown in the table.

SD1/SD2/SD3/SD4/SD5/SD6	20 µL CHO DNA qPCR MIX + 20 µL SD1/SD2/SD3/SD4/SD5/SD6
Workflow positive control	20 µL CHO DNA qPCR MIX + 20 µL Positive Control
Workflow Sample	20 µL CHO DNA qPCR MIX + 20 µL Sample
Workflow Spiked sample	20 µL CHO DNA qPCR MIX + 20 µL Recovery Control
Workflow Negative Control	20 µL CHO DNA qPCR MIX + 20 µL Negative Control
NTC (PCR negative control)	20 µL CHO DNA qPCR MIX + 20 µL PCR negative control

IMPORTANT! Vortex samples for 15-30 seconds before adding the samples to the PCR Plate, then tap to bring contents to the bottom of the wells.
If you prepared samples with the automated protocol, use a multichannel pipette to transfer the extracted sample.

9. See your software user guide for specific instructions. Place samples, NTCs, and standards in different quadrants of the plate.

Figure 2 Illustrates an example of a plate setup for only one sample, including all standards and controls. For accurate quantitative results, run triplicates for each PCR. Always run negative controls (NTC). To prepare a negative control (NTC),

replace the template DNA with the PCR Negative Control.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	SD6			Workflow positive control			Workflow Sample			Workflow Spiked sample		
C	SD5											
D	SD4											
E	SD3											
F	SD2											
G	SD1									Workflow Negative Control		
H										NTC (PCR negative control)		

Figure 2 Plate configuration proposal for one sample.

10. Use a film applicator to seal the plate with optical film, sthen briefly centrifuge with a miniplate centrifuge that is compatible with 96-well plates. Snap on the lids of the multi-wells, flick away the bubbles in the wells the briefly centrifuge with centrifuge.
11. Load the prepared 96-well plate into the PCR Instrument and start the run.

Note: Use reagents from the same lot for all reactions.

12. For the data analysis with the PCR Instrument, Abs Quant/2nd Derivative Max for All Samples is recommended. For more information, refer to the PCR Instrument Operator's Manual.

CHAPTER 6 Setup, run, and analyze samples with Software on the PCR Instrument

6.1 Use the kit with 7500 System SDS Software v1.5.1 to create the plate document, run the plate, and analyze the results

The following instructions apply only to the Applied Biosystems™ 7500 Fast instrument with SDS v1.x software. If you use a different instrument or software, refer to the applicable instrument or software documentation.

6.1.1 Create a plate document

CHO residual DNA assays are duplex assays, containing sample DNA and Internal Positive Control (IPC).

6.1.1.1 Plate document: settings

If you have run the assay frequently, you can use the table below to enter settings in Plate Document fields. If you are a new user, follow the detailed instructions in the following sections.

Summary of settings for the Plate Document		
In this field…		Use these settings
Detector	CHO	FAM™ dye (Select NFQ_MGB dye for quencher)
	IPC	ROX™ dyes (Select NFQ_MGB dye for quencher)
PCR	UDG[1]	Temp: 50°C Time: 2:00
PCR	Hold	Temp: 95°C Time: 5:00
PCR	Cycling (Standard Mode)	Cycles: 45 Temp: 95°C Time: 0:15 Temp: 60°C Time: 0:35 (acquisition)
Analysis	CHO	Automatic Baseline or Manual Baseline[2]. Automatic Baseline for IPC.

[1] UDG enzyme is used in this kit to avoid the contamination of amplification products.

[2] You can analyze the assay using Automatic or Manual Baseline, use the setting that yields the best standard curve.

6.1.1.2 Plate document: procedure

Use the SDS v1.x software on the Applied Biosystems™ 7500 Fast instrument to

perform this procedure.

1. In the template Assay dropdown list, select Absolute Quantification.
2. In the Run Mode dropdown list, select Standard 7500.
3. Enter resDNA CHO Template in the Plate name field, then click Next.
4. Click New Detector:

❖ Enter the name of the target cell line in the Name field.
❖ Select reporter FAM™ dye and quencher NFQ_MGB dye.
❖ Select a color for the detector, then click Create Another.

5. Click New Detector:

❖ Enter IPC in the Name field.
❖ Select reporter ROX™ dye and quencher NFQ_MGB dye.
❖ Select a color for the detector, then click OK.
❖ Select the detectors, then click Add>> to add the detectors to the document (plate).

6. Select ROX™ dye as the passive reference dye, then click Next.
7. Select the applicable set of wells for the samples, then select the target cell line and IPC detectors for each well. The following figure shows an example plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	SD6			Workflow positive control			Workflow Sample			Workflow Spiked sample		
C	SD5											
D	SD4											
E	SD3											
F	SD2											
G	SD1									Workflow Negative Control		
H										NTC (PCR negative control)		

8. Set tasks for each sample type by clicking on the Task Column dropdown list

❖ NTC: target cell line detector task = NTC
❖ Positive Control, test samples, Spiked samples: target DNA detector task = Unknown
❖ IPC = Unknown for all wells

9. Set up the standard curve:

❖ Select the wells.
❖ Assign the tasks (target DNA = Standard) and enter the appropriate Quantity for each set of triplicates.

Tube label	Row-wells	Task	Concentration(pg/μL)	Quantity (pg)
SD6	B-1,2,3	Standard	0.003	0.06
SD5	C-1,2,3	Standard	0.03	0.6
SD4	D-1,2,3	Standard	0.3	6
SD3	E-1,2,3	Standard	3	60
SD2	F-1,2,3	Standard	30	600
SD1	G-1,2,3	Standard	300	6000

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

- ❖ Set the thermal cycling reaction volume to 40 μ L.
- ❖ Set the reaction to Standard Mode.
- ❖ Set the temperature and the time as shown in the following table:

Step	UDG enzyme activation	AmpliTaq Gold™ enzyme activation	PCR	
	Hold	Hold	Cycle (45 Cycles)	
			Denature	Anneal/extend
Temp (°C)	50	95	95	60
Time (mm:sec)	2:00	5:00	0:10	0:30

Refer to the applicable 7500 Fast Real-Time PCR Systems instrument manual for additional information.

11. In the Analysis Settings window, enter the following settings, then click OK:

- ❖ Select Manual Ct.
- ❖ In Threshold, enter 10000.
- ❖ Select Automatic Baseline or Manual Baseline.

Note: You can analyze the assays using Automatic or Manual Baseline, use the setting that yields the best standard curve.

12. Select File►**Save as**, confirm that the file is named "resDNA_Template", then select **SDS Templates (*.sdt)** in the **Save as type** dropdown list and close the template plate document.

Note: You can reuse the plate template document whenever you run the assay.

13.Close the saved template file.

6.1.2 Run the plate

1. In the SDS software, select **File►New**, navigate to the **resDNA_Template** file (created in "Plate document: procedure"), then click Open.
2. In **Plate Name**, enter an appropriate experiment name, then click **Finish**.
3. Make any necessary changes to the test sample labels.
4. Select **Save As** to save the new experiment as an SDS experiment file.
5. Load the plate on the instrument.
6. Select the Instrument tab, save the document, then click Start to start the real-time qPCR run.

6.1.3 Analyze the results

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

1. Select the **Results** tab.
2. Select the **Amplification Plot** tab.
3. Verify the analysis settings, change as appropriate, then click **Analyze**.
4. Select the **Results tab►Standard Curve** tab, then verify the Slope, Intercept, and R2 values.
5. Right-click the **Standard Curve**, select **Export as JPEG**, then click **OK**. Alternatively, press **PrintScreen**, then paste the image in a WordPad file.

6. Select the **Report tab►Report**, then review the mean quantity and standard deviation for each sample.
7. Optional: Select **File►Export►Results**. In the **Save as type** drop-down list, select **Results Export Files (*.csv)**, then click **Save**.

Note: Refer to "Prepare the PCR plate" on page 16 for best practice.

6.2 Use the kit with the SLAN96S 8.2 software to Create the plate document, run the plate, and analyze the results

The SLAN 8.2.2 Program of SLAN®-96S Real-time PCR System is composed of three functional modules: Experiment, Project and Tools.
A project is a unique profile of SLAN Real-time PCR System, containing experiment type, channel information, PCR profile, analysis parameter, rules of judgment of results, etc. Usually a project is based on an experiment protocol, it provide a convenient and quick access to set experiments. Users can create or edit projects according to the experiment protocol, or contact the reagent manufacturer to acquire project files.

6.2.1 Create a progect file

Residual DNA assays are duplex assays, containing sample DNA and Internal Positive Control (IPC).

6.2.1.1 progect file: settings

If you have run the assay frequently, you can use the table below to enter settings in progect file fields. If you are a new user, follow the detailed instructions in the following sections.

Summary of settings for the Plate Document		
In this field…		Use these settings
Channel	CHO	FAM
	IPC	ROX
PCR	UDG[1]	Temp: 50°C Time: 2:00
PCR	Hold	Temp: 95°C Time: 5:00
PCR	Cycling (Standard Mode)	Cycles: 45 Temp: 95°C Time: 0:15； Temp: 60°C Time: 0:35, acquisition
Analysis	CHO	Automatic Baseline for CHO and IPC.

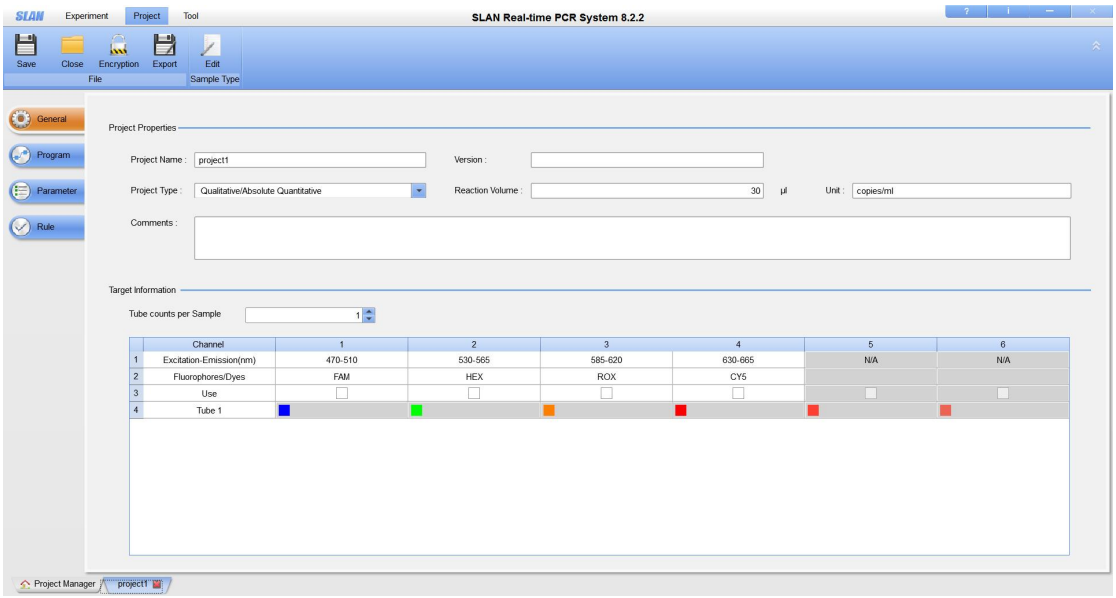
[1]UDG enzyme is used in this kit to avoid the contamination of amplification products.

6.2.1.2 Plate document: procedure

1. Double-click the SLAN8.2.2 software icon  on the desktop, or click Start>

Programs> SLAN Real-time PCR System 8.2.2 to enter the software.

2. Click **Create** button on the left of the Project Manager screen to open the project wizard.



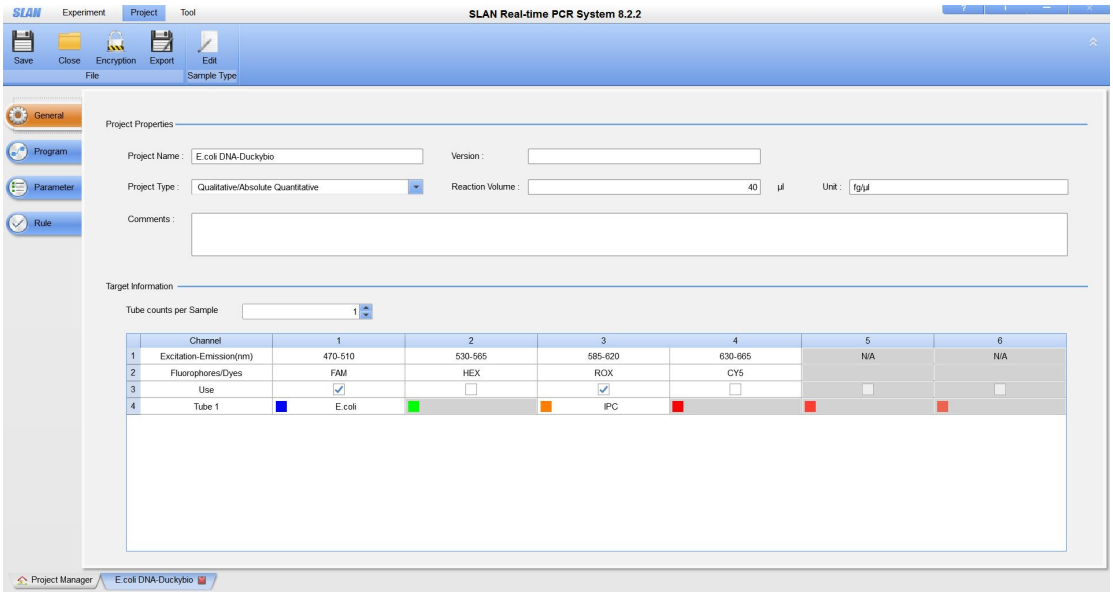
While the experiment is running, you cannot edit the Project being used by the experiment.

3. Click the General tab on the left and enter the general information for your project in the screen:

- ❖ Project Name: Enter a project name. Like CHO DNA-Duckybio. The string length of the name ranges from 1 to 50.
- ❖ Project Type: Select **Qualitative/Absolute Qualitative** in the Project Type drop-down list.
- ❖ Reaction Volume: Enter the reagent volume. The allowable reaction volume is 40µL.
- ❖ (Optional) Comments： Input note information for your project if necessary.

4. Input target information in Target Information area.

- ❖ Enter 1 in the Tube Couts per Sample
- ❖ Determine the FAM and ROX channels by selecting the checkbox ☑ below channels. Inter 1 in Tube1 below
- ❖ Enter target names like CHO and IPC. Please note that a target name must be exclusive.
- ❖ Check the right detection channels and enter target names. There should be at least one target for each detection channel.



5. Click on the Program tab to go to the thermal program screen.

❖ Insert Segment: Click on Insert Segment menu to add segment at the end of the currently selected segment. You can also insert segments through the right-click command Insert Segment. 2 segments will be need in this experment.

❖ Insert Step: Select a step in a segment, and then click Insert Step menu to insert a new step after the currently selected step.

❖ Modify Target Temperature: Click on the temperature box 

and enter a temperature, or drag the blue line below the temperature box to change temperature.

❖ Modify Holding Time: Click on the time box 

and enter the time;

❖ Set Fluorescence detection: click the box  and then the icon turns blue  , showing that “acquisition” of fluorescence will be performed at the current step. In a segment, only one step can be set for “acquisition” of fluorescence. A step with the holding time of less than 15 seconds does not support the acquisition of fluorescence.

❖ Modify Cycles: Click on the Cycle box 

below the segment2 and enter 45.

6. Click Save menu and then click Close menu to close the project.

6.2.2 Run the plate

1. Click “Experiment” to the Experiment Home screen, Experiment Home provides multiple shortcuts to creating or analyzing experiments, as described below:

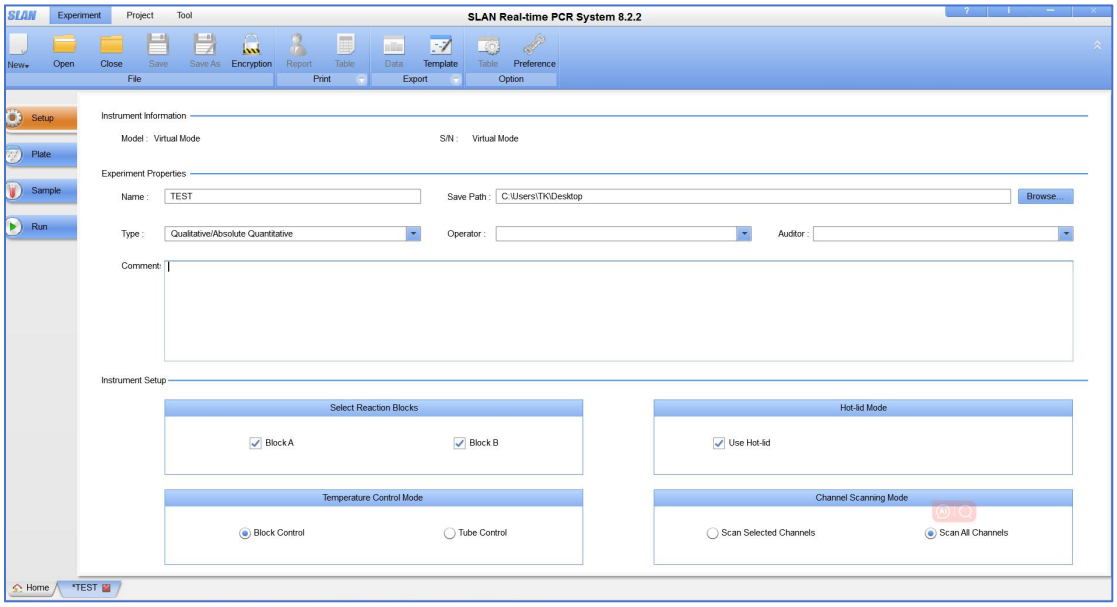
❖ Experiment Wizard: Create a new experiment by following the Experiment

Wizard.

- ❖ Latest Experiment: Create a new experiment with settings and plate from the latest experiment.
- ❖ Import Plate Layout: Run an experiment by importing an experiment configuration file in format of csv.
- ❖ Open Experiement: Open an experiment file for analysis.

2. Setup： Click “Experiment Wizard”, Click the Setup tab (Fig 4- 20) and enter the general information in the Experiment Information area:

- ❖ In the field of Experiment Name, enter the name of the experiment.
- ❖ Select the Qualitative/Absolute Qualitative type of experiment in the Experiment Type drop-down list.
- ❖ Modify the path in which the file is saved in the “Save Path” field.
- ❖ Select “Use Hot-lid” in the area of Hot-lid Mode.
- ❖ In Channel Scanning Mode area, select All Channels scanning mode.



3. Setup Plate：Click Plate tab to enter the screen for well defining. Choose wells to be edited in the Well Selector. In case of multi-tube samples, the number of wells you need to select should be at least greater than the well numbers of a sample.

- ❖ Select “CHO DNA-Duckybio” project created before from the project list in the top right of the screen.

4. Edit the sample type and property in Step 3:

- ❖ NTC: target cell line detector task = NTC
- ❖ Positive Control, test samples, Spiked samples: target DNA detector task = Unknown
- ❖ IPC = Unknown for all wells

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	SD6			Workflow positive control			Workflow Sample			Workflow Spiked sample		
C	SD5											
D	SD4											
E	SD3											
F	SD2											
G	SD1									Workflow Negative Control		
H										NTC (PCR negative control)		

5. Set up the standard curve:

❖ Select the wells.

❖ Assign the tasks (target DNA = Standard) and enter the appropriate Quantity for each set of triplicates.

Tube label	Row-wells	Task	Concentration(pg/μL)	Quantity (pg)
SD6	B-1,2,3	Standard	0.003	0.06
SD5	C-1,2,3	Standard	0.03	0.6
SD4	D-1,2,3	Standard	0.3	6
SD3	E-1,2,3	Standard	3	60
SD2	F-1,2,3	Standard	30	600
SD1	G-1,2,3	Standard	300	6000

6. Run an Experiment

❖ Click Run tab to enter the Run screen, on which such information as test samples and projects is displayed. Place sample tubes in the reaction block of the instrument according to the sequence of the Well Selector. Click the 

button to run the experiment.

❖ Monitor Running: The real time temperature curve and operation status are displayed on the top right of the experiment Run screen while the real-time curve is shown at the bottom right.

❖ When the experiment is finished, the reaction module is cooled and the hot lid is opened automatically. The software will automatically save results and redirect to the Analysis tab.

❖ Remove the sample tubes from the PCR instrument and dropped into a fresh hypochlorous acid solution for disinfection to prevent contamination of PCR amplification products.

Note: Do not open the sample tube at any time!!!

6.2.3 Analyze the results

1. Adjusting Analyze Parameters

❖ The instrument collects real-time fluorescence data during the experiment; when the experiment is over, the software automatically processes raw data in accordance with the analysis parameters of the project.

❖ Click Open Experiments button in the Experiment Home screen.

- The most frequently used analysis parameters of the current experiment is displayed in the analysis parameter table, which is below the Sample Information Table.
- Click the Analysis Parameters memu in the drop-down menu of Analysis to view all the analysis parameters. Choose Relative fluorescence method in the drop-down menu of Normolization algorithm. Choose Quantitative in the drop-down menu of Analysis Type of each Target. Click the Analysis menu to Analysis the result.
- Click Data in the menu bar to export data of this experiment (Fig 4- 37). Data can be saved in formats of XLSX, CSV or TXT.

CHAPTER 7 Result interpretation

7.1 Threshold line setting

The threshold line should be adjusted based on instrument noise to just exceed the vertex of the amplification curve of the CHO negative control sample. If the automatic threshold line of the instrument exceeds this point, it can still be used.

7.2 Internal Positive Control interpretation

For negative results, internal control Ct value should be ≤30; for positive results, competition inhibition may cause no or poor values for internal controls.

7.3 Experiment establishment conditions

FAM channel shows no Ct value or a Ct value >36 in NTC and NCS samples; standard curve has a linear correlation coefficient R^2 >0.99.

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

CHAPTER 8 Good laboratory practices

8.1 Work area setup and lab design

The sensitivity of this kit (and other PCR-based tests) enables amplification of minute quantities of DNA, necessitating precautions to avoid contamination of samples yet to be amplified .

Process samples carefully to prevent contamination by human DNA. Wear gloves at all times and change them frequently. Close sample tubes when not in use. Limit aerosol dispersal by handling sample tubes and reagents carefully.

8.2 Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
- Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
- Sample preparation and reaction setup.
- Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontaminations olution.

8.3 Avoiding false positives due to cross-contamination

To avoid false positives due to cross-contamination:

- Do not open tubes after amplification.
- Use different sets of pipettors when pipetting negative control, unknown, and positive control samples.

CHAPTER 9 Troubleshooting

Problem	Cause	Recommendation
Fluorescence intensity varies.	Some of the reagent is still in the upper part of the microwell or an air bubble is trapped in the microwell.	Repeat centrifugation, but allow sufficient centrifugation time so all reagent is at the bottom of the microwell and air bubbles are expelled.
	Skin oils or dirt on the surface of the microwell plate.	Always wear gloves when handling the multiwell plate.
Fluorescence intensity is very low.	Low concentration or deterioration of dyes in the reaction mixtures because dye was not stored properly.	1. Keep dye-labeled reagents away from light. 2. Store the reagents at -15 to -25°C and avoid repeated freezing and thawing.
	Poor PCR efficiency (reaction conditions not optimized).	Always run a positive control along with your samples.
	DNA is degraded during isolation or improper storage.	1. If possible, check DNA quality. 2. Store DNA samples at -15 to -25°C.
	Pipetting errors and/or omitted reagents.	1. Check for missing reagents. 2. Check the pipetting procedure.
	Impure sample material inhibits reaction.	Dilute sample 1:10 and repeat the analysis.
Negative control sample gives a positive signal.	Contamination	Remake all critical reaction mixes. Be sure to use special pre-PCR setup working areas.
Slope for the standardcurve is outside the typical range, or R^2 value is significanthless than 0.99	When applying detectors for standards, the Task and Quantity were applied to the wrong detector. The incorrect Quantity wasentered. Adjust baseline settings. Poor standard curve preparation technique (forgot to mix,inaccurate pipetting)	1. In the SoS sofware, from the plate document,double-click a well containing a DNA standard toview the Well lnspector. 2. Ensure that the correct Task and Quantity are2applled to the correct detector, then reanakzeCompare std curve statistics using autobaseline omanual baseline. The upper limit of the manualbaseline setting must be 2 cycles before uptick inamplification. Verify in Rn vs c, linear view.

Δ Rn and Ct values are inconsistent with replicates	Evaporation of reaction mixture from some wells occurred because the optical adhesive cover was not correctly sealed to the reaction plate or due to over-drying the eluates in PrepSEQ™.	1. Select the Component tab. Confirm that affected wells generated significantly less fluorescence than unaffected replicates. 2. Check the amount of solution in each well of the reaction plate. Confirm that the wells affected by evaporation contained less solution than unaffected wells, and corresponded with the inconsistent results. 3. For subsequent runs, ensure that the optical adhesive cover is correctly sealed to the reaction plate.
	Incorrect volume of PCR reaction mix was added to some reactions.	1. Select the Component tab. Confirm that affected wells generated significantly less fluorescence than unaffected replicates. 2. Select the Spectra tab. Confirm that the wells with the incorrect volume of PCR reaction mix generated significantly different amounts of fluorescence than the unaffected wells. 3. For subsequent runs, ensure the correct volume of PCR reaction mix.
Jagged amplification plots	Weak lamp or incorrect replacement.	Replace the lamp or ensure that the existing replacement is correct.
No defined amplification plots	An incorrect detector was selected on the amplification plot. or An incorrect detector was applied to the reactions when setting up the plate document.	1. Ensure that the correct detector was selected on the amplification plot. 2. If the correct detector was not selected, then in the plate document, double-click a well to view the Well Inspector, verify that the detector settings are correct, and reanalyze.
Abnormal ΔRn values or negative ΔRn values	Incorrect passive reference was selected when setting up the plate document.	1. From the plate document, double-click a well to view the Well Inspector. 2. Ensure that ROX™ dye was selected as the Passive Reference.

Standard curve assays is outside of the 90–110% efficiency range	Incomplete vortexing of low level standards.	Repeat reactions, ensuring that samples and standards are vortexed for 15-30 seconds.
Wide variance of Ct values of samples	Incomplete vortexing of samples.	
The Ct Values of the startand curve is is abnormal	The disinfectant affected the PCR amplification	Do not use disinfectants during Sample preparation experiments so as not to affect the results.

CHAPTER 10 Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

·Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.

·Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).

·Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).

·Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.

·Handle chemical wastes in a fume hood.

·Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)

·After emptying a waste container, seal it with the cap provided.

·Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.

·Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

·IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! Reagent and Waste Bottle Safety. Reagent and waste bottles can crack and leak. Each bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.