resDNASEQ™ Quantitative DNA Kits

USER GUIDE

for use with:
resDNASEQ™ Quantitative CHO DNA Kit
resDNASEQ™ Quantitative *E. coli* DNA Kit
resDNASEQ™ Quantitative HEK293 DNA Kit
resDNASEQ™ Quantitative Human DNA Kit
resDNASEQ™ Quantitative Vero DNA Kit
resDNASEQ™ Quantitative *Pichia* DNA Kit
resDNASEQ™ Quantitative NS0 DNA Kit
resDNASEQ™ Quantitative MDCK DNA Kit

Catalog Numbers 4402085, 4458435, A46014, A26366, A41797, 4464336, 4458441, 4464335
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For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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

Revision history: Pub. No. 4469836

Revision	Date	Description
F	17 December 2019	Update to include the resDNASEQ™ Quantitative HEK293 DNA Kit (Cat. No. A46014).
E	21 October 2019	Update to include run and analysis information for AccuSEQ™ Real-Time PCR Software v3.0.

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Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The resDNASEQ™ Quantitative DNA Kits are used to quantitate host-cell residual DNA from CHO, *E. coli*, HEK293, Human, Vero, *Pichia*, NS0, and MDCK 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 *PrepSEQ™ Residual DNA Sample Preparation Kit User Guide* (Pub. No. 4469838).

The resDNASEQ™ Quantitative DNA Kits use TaqMan™ quantitative PCR to perform rapid, specific quantitation of sub-picogram levels of residual host-cell DNA. The assay is accurate and reliable across a broad range of sample types, from in-process samples to final product.

To generate the standard curve used to quantitate the DNA in test samples, the CHO, Vero, NS0, and MDCK assays require six dilutions (from 30 fg to 3 ng per reaction) and the *E. coli*, HEK293, Human, and *Pichia* assays require five dilutions (300 fg to 3 ng per reaction). Control DNA for standard curve generation is included in the kits. In addition, the kits use an internal positive control (IPC) to evaluate the performance of each PCR reaction.

Contents

Table 1 resDNASEQ™ Quantitative CHO DNA Kit (Cat. No. 4402085)

Contents	Amount	Storage
resDNASEQ™ CHO DNA Control		
CHO DNA Control, 30 ng/µL	40 µL	–25°C to –15°C
DNA Dilution Buffer (DDB)	7 mL	–25°C to –15°C 2–8°C after first use
resDNASEQ™ CHO Real-Time PCR Reagents		
2X Environmental Master Mix	2 × 0.75 mL	–25°C to –15°C before first use, protect from light 2–8°C after first use, protect from light
10X CHO DNA Real-Time PCR Assay Mix	300 µL	–25°C to –15°C, protect from light
Negative Control (water)	1.0 mL	–25°C to –15°C before first use 2–8°C after first use

Table 2 resDNASEQ™ Quantitative *E. coli* DNA Kit (Cat. No. 4458435)

Contents	Amount	Storage
Box 1, resDNASEQ™ Real-Time PCR Reagents		
2X Environmental Master Mix	2 × 0.75 mL	–25°C to –15°C before first use, protect from light 2–8°C after first use, protect from light
Negative Control (water)	1.0 mL	–25°C to –15°C before first use 2–8°C after first use
10X *E. coli* DNA Assay Mix	300 µL	–25°C to –15°C, protect from light
Box 2, resDNASEQ™ *E. coli* DNA Control		
E. coli DNA Control, 30 ng/µL	40 µL	–25°C to –15°C
DNA Dilution Buffer (DDB)	7 mL	–25°C to –15°C before first use 2–8°C after first use

Table 3 resDNASEQ™ Quantitative HEK293 DNA Kit (Cat. No. A46014)

Contents	Amount	Storage
resDNASEQ™ HEK293 DNA Control		
HEK293 DNA Control, 30 ng/µL	40 µL	–25°C to –15°C
DNA Dilution Buffer (DDB)	7 mL	–25 to –15°C before first use 2–8°C after first use
resDNASEQ™ HEK293 DNA Real-Time PCR Reagents		
2X Environmental Master Mix	2 × 0.75 mL	–25°C to –15°C before first use, protect from light 2–8°C after first use, protect from light
10X HEK293 Assay Mix	300 µL	–25°C to –15°C, protect from light
Negative Control (water)	1.0 mL	–25°C to –15°C before first use 2–8°C after first use

Table 4 resDNASEQ™ Quantitative Human DNA Kit (Cat. No. A26366)

Contents	Amount	Storage
resDNASEQ™ Human DNA Control		
Human DNA Control, 30 ng/µL	40 µL	–25°C to –15°C
DNA Dilution Buffer (DDB)	7 mL	–25 to –15°C before first use 2–8°C after first use
resDNASEQ™ Human Real-Time PCR Reagents		
2X Environmental Master Mix	2 × 0.75 mL	–25°C to –15°C before first use, protect from light 2–8°C after first use, protect from light
10X Human DNA Assay Mix	300 µL	–25°C to –15°C, protect from light
Negative Control (water)	1.0 mL	–25°C to –15°C before first use 2–8°C after first use

Table 5 resDNASEQ™ Quantitative MDCK DNA Kit (Cat. No. 4464335)

Contents	Amount	Storage
Box 1, resDNASEQ™ Real-Time PCR Reagents		
2X Environmental Master Mix	2 × 0.75 mL	–25°C to –15°C before first use, protect from light 2–8°C after first use, protect from light
Negative Control (water)	1.0 mL	–25°C to –15°C before first use 2–8°C after first use
10X MDCK DNA Assay Mix	300 µL	–25°C to –15°C, protect from light
Box 2, resDNASEQ™ MDCK DNA Control		
MDCK DNA Control, 30 ng/µL	40 µL	–25°C to –15°C
DNA Dilution Buffer (DDB)	7 mL	–25°C to –15°C before first use 2–8°C after first use

Table 6 resDNASEQ™ Quantitative NS0 DNA Kit (Cat. No. 4458441)

Contents	Amount	Storage
Box 1, resDNASEQ™ Real-Time PCR Reagents		
2X Environmental Master Mix	2 × 0.75 mL	–25°C to –15°C before first use, protect from light 2–8°C after first use, protect from light
Negative Control (water)	1.0 mL	–25°C to –15°C before first use 2–8°C after first use
10X NS0 DNA Assay Mix	300 µL	–25°C to –15°C, protect from light
Box 2, resDNASEQ™ NS0 DNA Control		
NS0 DNA Control, 30 ng/µL	40 µL	–25°C to –15°C
DNA Dilution Buffer (DDB)	7 mL	–25°C to –15°C before first use 2–8°C after first use

Table 7 resDNASEQ™ Quantitative *Pichia* DNA Kit (Cat. No. 4464336)

Contents	Amount	Storage
Box 1, resDNASEQ™ Real-Time PCR Reagents		
2X Environmental Master Mix	2 × 0.75 mL	–25°C to –15°C before first use, protect from light 2–8°C after first use, protect from light
Negative Control (water)	1.0 mL	–25°C to –15°C before first use 2–8°C after first use
10X *Pichia* DNA Assay Mix	300 µL	–25°C to –15°C, protect from light
Box 2, resDNASEQ™ *Pichia* DNA Control		
Pichia DNA Control, 30 ng/µL	40 µL	–25°C to –15°C
DNA Dilution Buffer (DDB)	7 mL	–25°C to –15°C before first use 2–8°C after first use

Table 8 resDNASEQ™ Quantitative Vero DNA Kit (Cat. No. A41797)

Contents	Amount	Storage
Box 1, resDNASEQ™ Real-Time PCR Reagents		
2X Environmental Master Mix	2 × 0.75 mL	–25°C to –15°C before first use, protect from light 2–8°C after first use, protect from light
Negative Control (water)	1.0 mL	–25°C to –15°C before first use 2–8°C after first use
10X Vero DNA Assay Mix	300 µL	–25°C to –15°C, protect from light
Box 2, resDNASEQ™ Vero DNA Control		
Vero DNA Control, 30 ng/µL	40 µL	–25°C to –15°C
DNA Dilution Buffer (DDB)	7 mL	–25°C to –15°C before first use 2–8°C after first use

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instrument	
QuantStudio™ 5 Real-Time PCR System with AccuSEQ™ Real-Time PCR Software v3.0	Contact your local sales representative.
Consumables	
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 20 plates, 0.1-mL wells; for use with Applied Biosystems™ 7500 Fast Real-Time PCR System	4346906
MicroAmp™ 96-Well Base	N8010531, 10 bases
MicroAmp™ Optical Adhesive Film	4311971, 100 covers 4360954, 25 covers
MicroAmp™ Adhesive Film Applicator	4333183, 5 applicators
Miscellaneous items	
Disposable gloves	Major lab supplier (MLS)
Pipettes	MLS
Aerosol-resistant micropipette tips	MLS
For the PCR plate: Fisher Scientific™ Mini Plate Spinner Centrifuge, 120- or 230-volt	14-100-143 (120-volt), 14-100-141 (230-volt)
Nonstick, RNase*Zap*™-free Microfuge Tubes, 1.5-mL (1 box; 250 tubes/box)	AM12450

Workflow

Prepare the control DNA serial dilutions
(page 12)

▼

Prepare the PCR reaction mix
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▼

Prepare the PCR plate
(page 15)

▼

AccuSEQ™ Software v3.0 for the QuantStudio™ 5 Instrument

Create a resDNASEQ™ experiment
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Start the run
(page 20)

▼

Analyze the results
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Prepare the control DNA serial dilutions for the standard curve

Guidelines for standard dilutions

- Prepare the standard curve and the test samples in different areas of the lab.
- Use different sets of pipettors for test sample preparation and for standard curve preparation and aliquoting to avoid cross-contamination of test samples.
- Vortex each tube to mix the contents thoroughly before each dilution step.
- Briefly centrifuge to collect all the liquid at the bottom before making the next dilution.

Prepare the control DNA serial dilutions

1. Label nonstick 1.5-mL microfuge tubes: **NTC, SD1**, **SD2**, **SD3**, **SD4**, **SD5**.
   For CHO, Vero, MDCK, and NS0 kits, label an additional tube with **SD6**.
2. Add 50 µL of DNA Dilution Buffer (DDB) to tube NTC. Put aside.
3. Add 990 µL of DNA Dilution Buffer (DDB) to tube SD1.
4. Add 450 µL of DNA Dilution Buffer (DDB) to tubes SD2, SD3, SD4, SD5, and (for CHO, Vero, MDCK, and NS0 only) SD6.
5. Remove the tube of DNA control (30 ng/µL) from the freezer.
6. After the DNA thaws, vortex it gently for 2 seconds, then briefly centrifuge.
7. Perform the serial dilutions:
   a. Add 10 µL of the DNA control to the tube that is labeled SD1, then vortex thoroughly and briefly centrifuge.
   b. Transfer 50 µL of the DNA from tube SD1 to tube SD2, then vortex thoroughly and briefly centrifuge.

c. Continue to transfer 50 µL of DNA from the previous dilution tube to the next dilution tube until you add DNA to tube SD5 (*E. coli*, HEK293, Human, and *Pichia*) or SD6 (CHO, Vero, MDCK, and NS0). Final dilutions are shown in the table. After each transfer, vortex thoroughly, then centrifuge briefly.

Serial dilution (SD) tube	Dilution	pg DNA/reaction (10 µL of the diluted DNA used in final 30 µL of PCR reaction)
Control	DNA control tube	300,000 pg
SD 1	10 µL DNA control + 990 µL DDB	3,000 pg
SD 2	50 µL SD1 + 450 µL DDB	300 pg
SD 3	50 µL SD2 + 450 µL DDB	30 pg
SD 4	50 µL SD3 + 450 µL DDB	3 pg
SD 5	50 µL SD4 + 450 µL DDB	0.3 pg
SD 6 (for CHO, Vero, MDCK, and NS0 only)	50 µL SD5 + 450 µL DDB	0.03 pg

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

Prepare the PCR reaction mix

1. Determine the number of reactions needed for the controls and test samples that you will quantify.

2. Thaw all kit reagents completely at room temperature, thoroughly mix reagents, and briefly centrifuge.

3. Prepare a PCR reaction mix using the reagents and volumes shown in the table below.
   - Multiply the PCR reaction volume for one reaction (30 µL) by the number of reactions that you need to run.
   - Use 10% excess volume to compensate for pipetting losses.

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

Kit reagents	Volume for 1 30-µL reaction	Volume for 36 30-µL reactions (includes 10% overage)
Negative Control (water)	2 µL	79.2 µL
10X DNA assay mix appropriate for the cell line being tested	3 µL	118.8 µL
2X Environmental Master Mix	15 µL	594 µL
DNA template	10 µL	Add DNA template to each well separately, not as part of Master Mix
Total	**30 µL**	**792 µL**

Prepare the PCR plate

Plate setup differs slightly for each AccuSEQ™ System. See your software user guide for specific instructions. Place samples, NTCs, and standards in different quadrants of the plate.

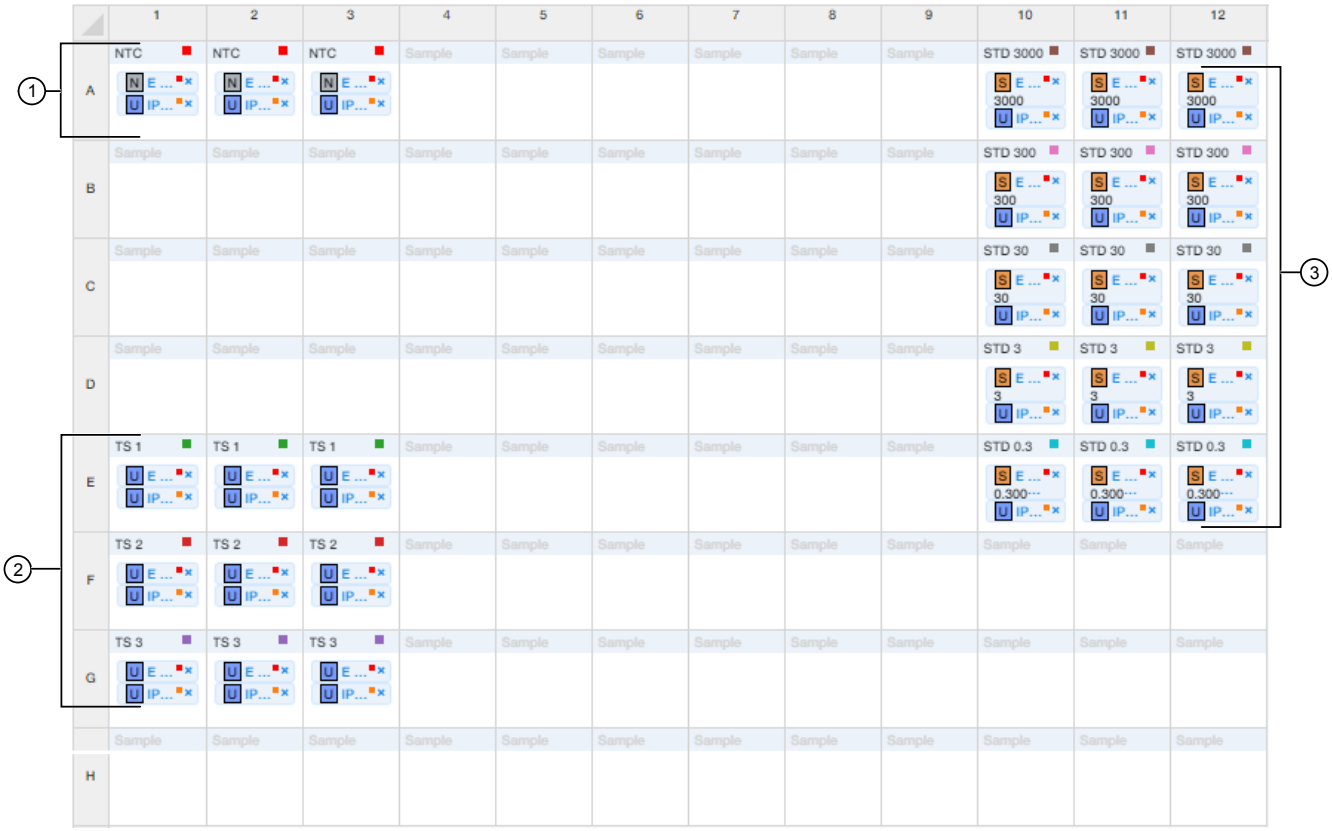


Figure 1 Default plate setup in the AccuSEQ™ Real-Time PCR Software v3.0

① No template controls
② Samples
③ Standard curve

1. Add 20 µL PCR reaction mix to each well.
2. Add 10 µL of PCR NTC to the appropriate wells.
3. Add 10 µL each of extracted sample DNA to the appropriate wells.

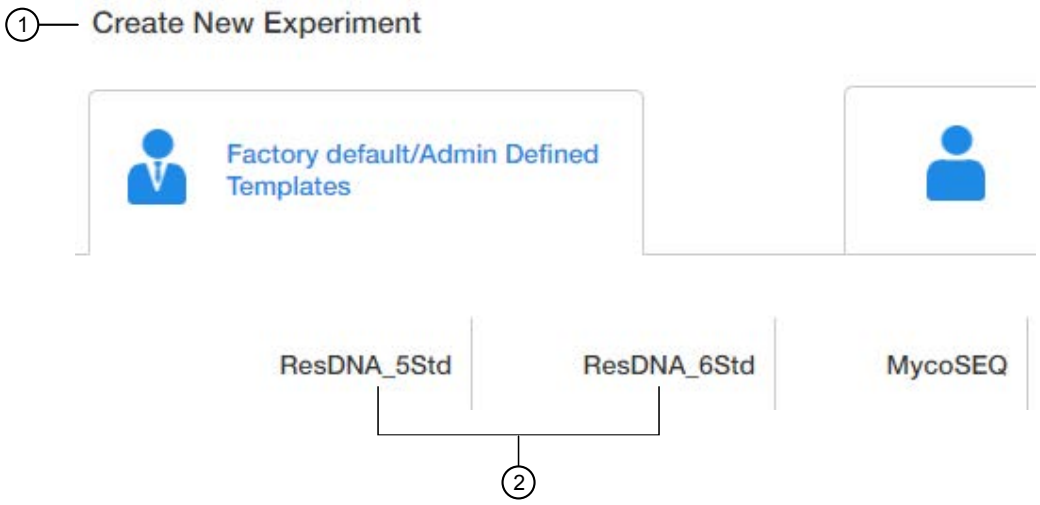
   Note: If you prepared samples with the automated protocol, use a multichannel pipette to transfer the extracted sample.
4. Add 10 µL of standard dilutions to the appropriate wells.

   Note: Use different sets of pipettors to dispense test sample and standard curve dilutions to avoid cross-contamination of test samples.
5. Use a film applicator to seal the plate with optical film, then briefly centrifuge with a miniplate centrifuge that is compatible with 96-well plates.

Setup, run, and analyze samples with AccuSEQ™ Software on the QuantStudio™ 5 Real-Time PCR Instrument

Create a resDNASEQ™ experiment

1. In the **Home** screen, click the **Factory default/Admin Defined Template** tab, then select a **resDNASEQ** template.



① **Factory default/Admin Defined Template** tab
② **resDNASEQ** template (ResDNA_5Std or ResDNA_6Std)

Template	Assays
5 standards (_5Std)	*E. coli*, HEK293, Human, and *Pichia*
6 standards (_6Std)	CHO, Vero, MDCK, and NS0

2. In the **Experiment Properties** pane of the **Setup** tab:
   a. (*Optional*) Change the system-generated name of the experiment.
   b. (*Optional*) Enter the plate **Barcode**, then add **Comments**.
   Default resDNASEQ™ settings (cannot be changed)
   - **Experiment Type** is **Quantitation-Standard Curve**
   - **Chemistry** is **TaqMan™ Reagents**
   - **Ramp Speed** is **Standard - 2hrs**
   c. Click **Next**.

3. In the **qPCR Method** pane of the **Setup** tab, view the default volume and cycling conditions (cannot be changed).

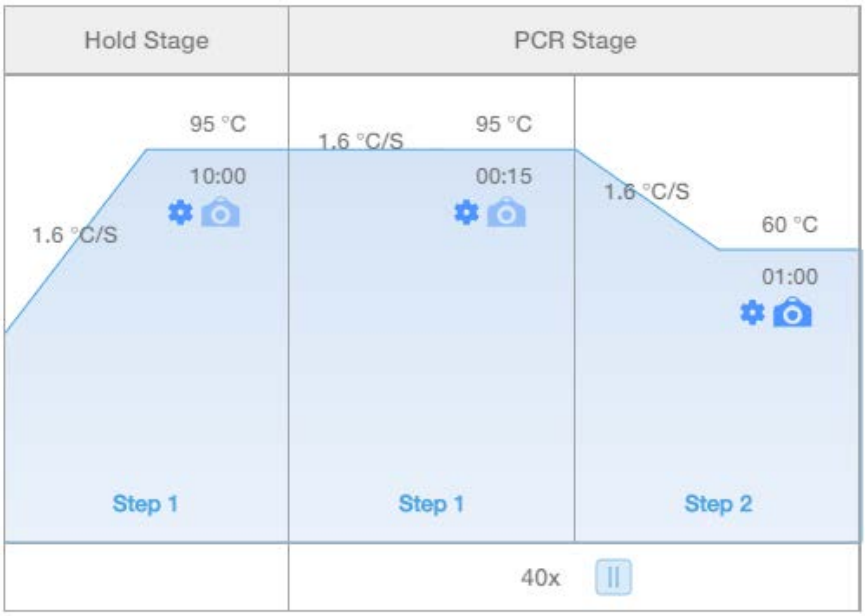
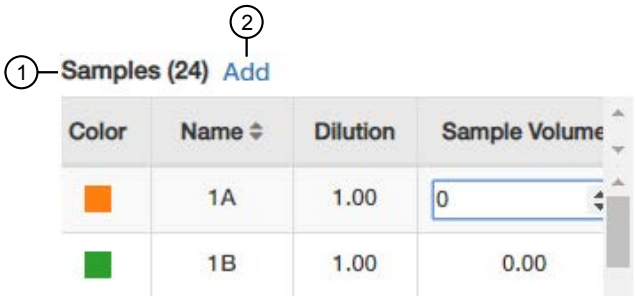


Figure 2 resDNASEQ™ template default cycling conditions

4. Click **Next**.

5. In the **Samples** pane of the **Setup** tab, enter the sample **Name** and **Dilution**. **Sample Volume** is not applicable. Add additional **Samples** if needed.

IMPORTANT! Do not change the **Targets**.



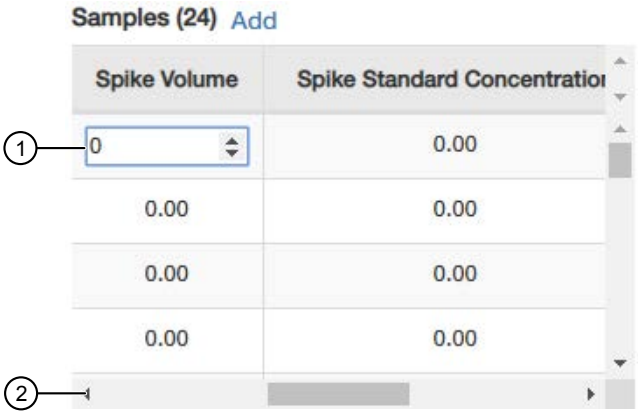
① **Samples** pane
② **Add**—adds additional samples

6. In the **Samples** pane of the **Setup** tab, scroll to the right, then enter the spike information.

   For more information on plate setup, see the *AccuSEQ™ Real-Time PCR Software v3.0 User Guide* (Pub. No. 100084348).

   - **Sample Volume**—not applicable; leave as default (0).
   - **Spike Volume**—volume of DNA added to the PCR (set to 10).
   - **Spike Standard Concentration**—expected spike amount per reaction (for example, 10pg).
   - **Reference**—the non-spiked sample; the mean quantity of reference is subtracted during % recovery calculation.
   - **Spike Input**—automatically calculated (double check if correct).

     Note: If incorrect, be sure **Spike Volume** is set to 10 and **Spike Standard Concentration** is the expected pg spike per PCR reaction.
   - (Optional) **Comments**
   - **Protein Concentration**—Drug substance protein concentration (if Total DNA in pg DNA/mg Protein is required).



① Textbox—type in the value, or use the up and down arrows
② Scroll bar—scroll to find the spike parameter

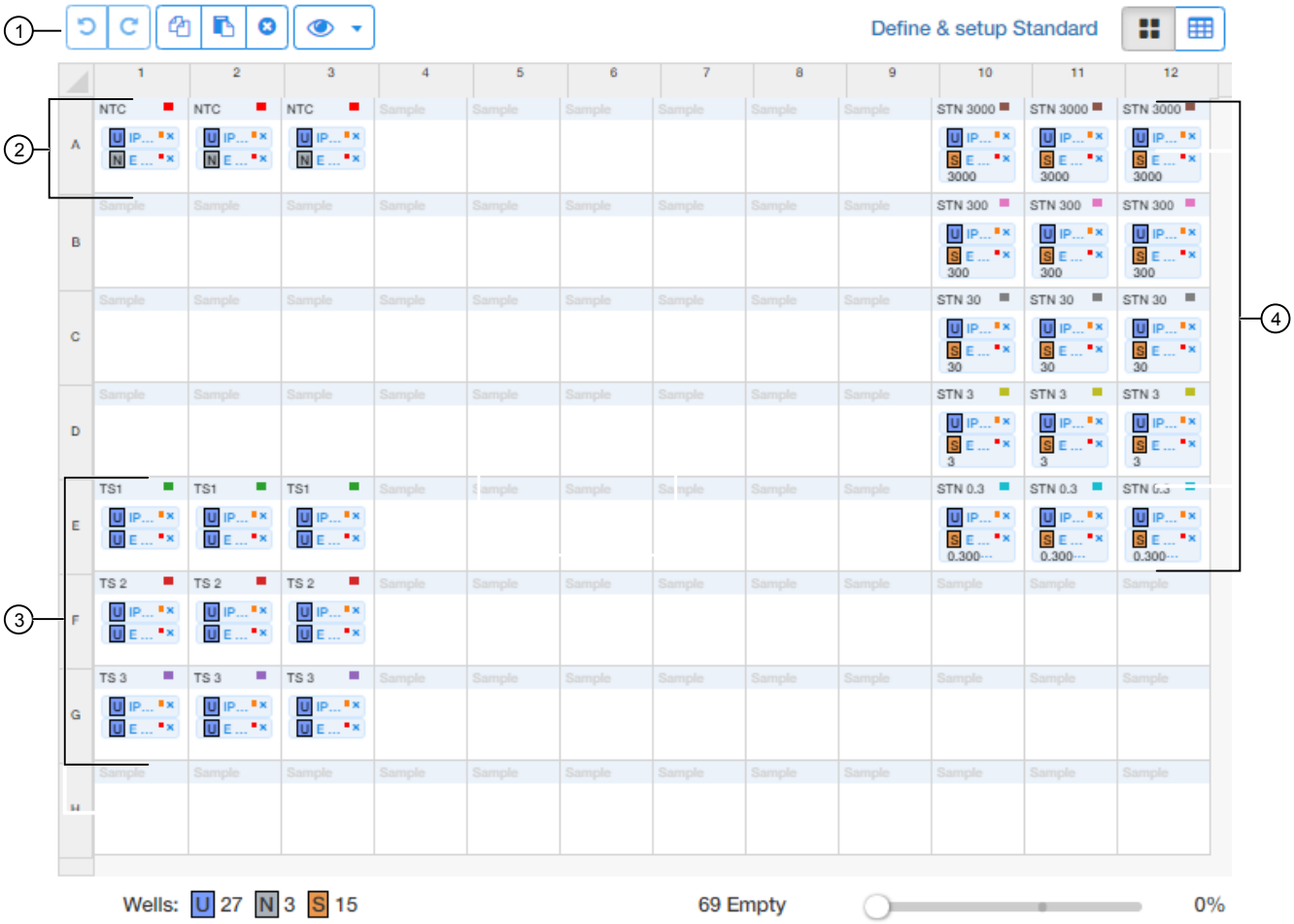


Figure 3 resDNASEQ™_5Std template default sample plate layout

① Toolbar (in order: Undo, Redo, Copy, Paste, Delete, View)
② 3 No Template Control (NTC) samples
③ 3 default **Samples**
④ Standard curve dilutions (S) in triplicate

7. Click **Next**.
   The **Run** tab is displayed.

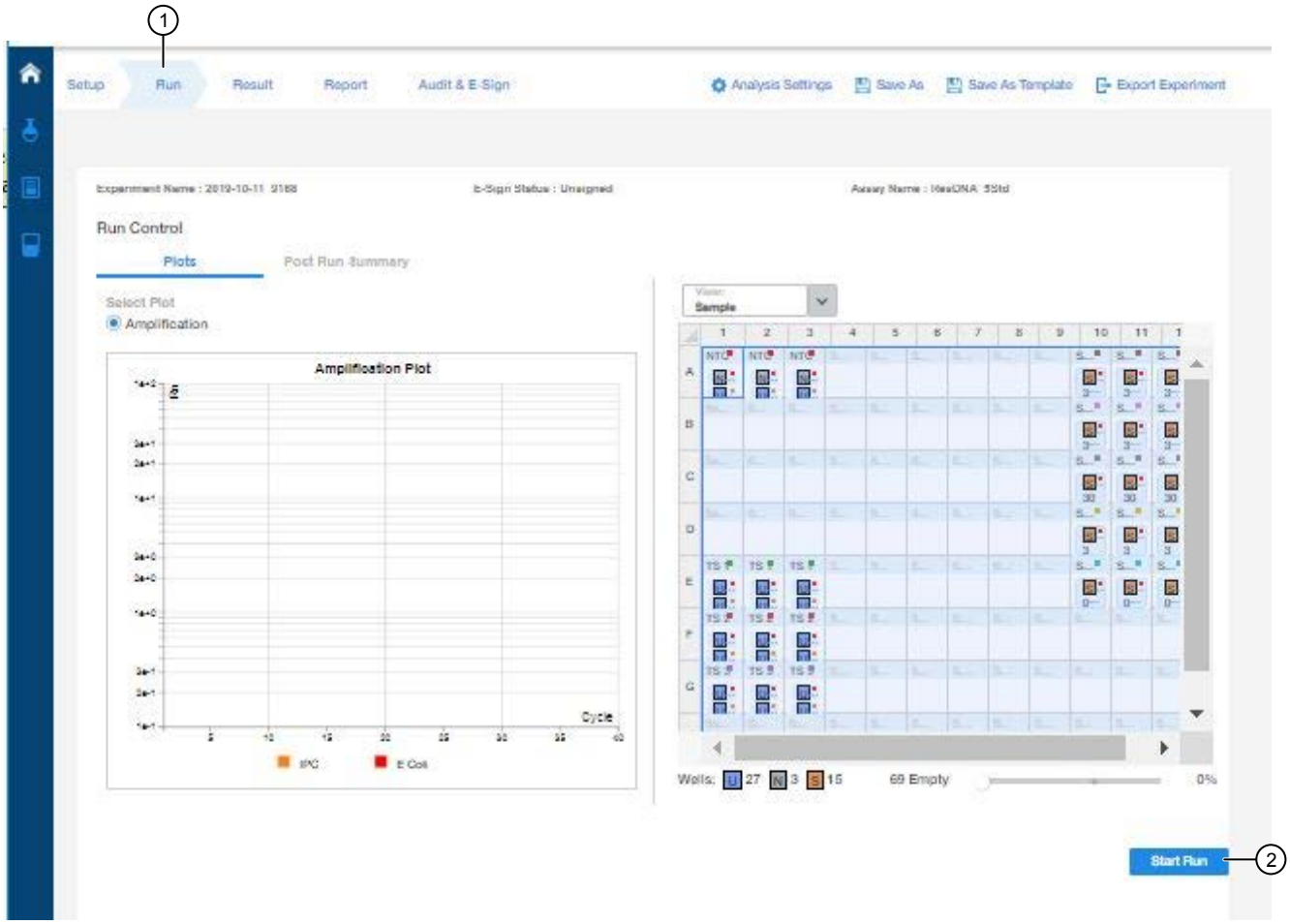
8. Experiments are auto-saved in the software. To save, exit the experiment. The software prompts you to save changes. Click **Yes**.

   Note: Clicking **Save As** will create a copy of the experiment.

Start the run

Start the run in the AccuSEQ™ Software.

Option	Description
If the experiment is open	Click **Start Run**.
If the experiment is closed	1. Open the experiment. 2. Click the **Run** tab. 3. Click **Start Run**.



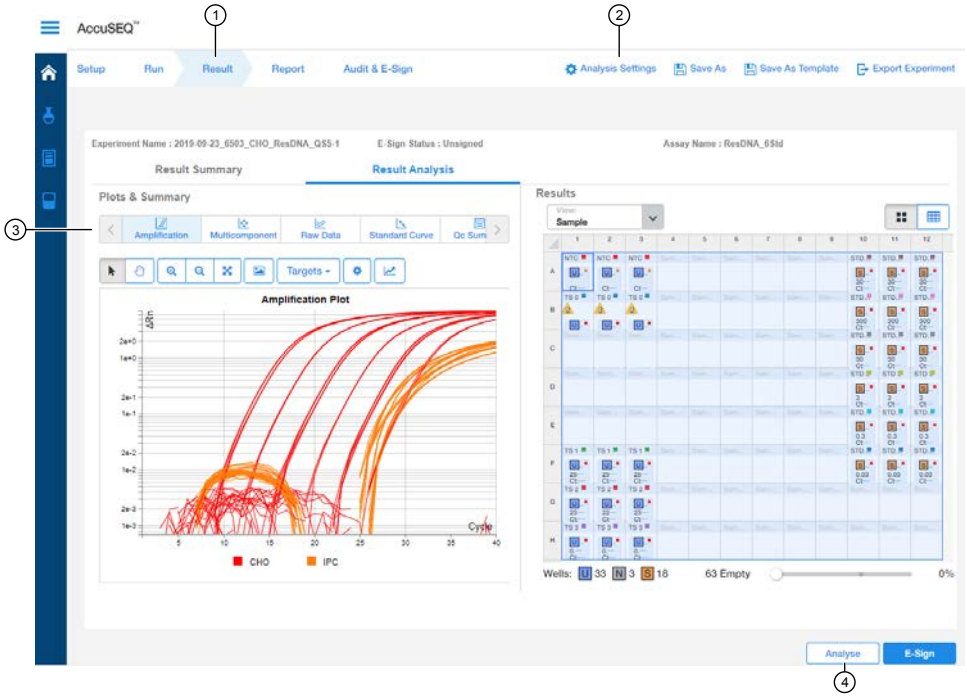
① **Run** tab
② **Start Run** button

A message stating **Run has been started successfully** is displayed when the run has started.

Analyze the results

After the qPCR run is finished, use the following general procedure to analyze the results. For more detailed instructions see the *AccuSEQ™ Real-Time PCR Software v3.0 User Guide* (Pub. No. 100084348).

1. In the AccuSEQ™ Real-Time PCR Software, open your experiment, then navigate to the **Result** tab.



① **Result** tab
② **Analysis Settings**
③ Plot horizontal scrollbar
④ **Analyze** button

2. In the **Result Analysis** tab, review the Amplification Curve plots for amplification profiles in the controls, samples, and the standard curve.

3. In the **Result Analysis** tab, review the **QC Summary** for any flags in wells.

4. In the **Result Analysis** tab, review the **Standard Curve** plot. Verify the values for the Slope, Y-intercept, R2, and Efficiency.

5. (*Optional*) Navigate to the **Report** tab to generate a report of the experiment, or to export results.

A

Troubleshooting

Observation	Possible cause	Action
Slope for the standard curve is outside the typical range, or R_2 value is significantly less than 0.99.	When applying detectors for standards, the Task and Quantity were applied to the wrong detector. *or* The incorrect Quantity was entered. *or* Adjust baseline settings. *or* Poor standard curve preparation technique (forgot to mix, inaccurate pipetting).	1. In the SDS software, from the plate document, double-click a well containing a DNA standard to view the Well Inspector. 2. Ensure that the correct Task and Quantity are applied to the correct detector, then reanalyze. 3. Compare std curve statistics using autobaseline or manual baseline. The upper limit of the manual baseline setting must be 2 cycles before uptick in amplification. Verify in Rn vs C_t linear view.
Δ Rn and C_t 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.

Observation	Possible cause	Action
Δ Rn and C_t values are inconsistent with replicates	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 make sure 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. Confirm 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 ΔR_n 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™ was selected as the Passive Reference.

B Use the kit with the 7500 Fast Real-Time PCR Instrument and AccuSEQ™ software v2.x

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instrument	
7500 Fast Real-Time PCR System with AccuSEQ™ software v2.x	Contact your local sales representative
Generic consumables	
Disposable gloves	MLS
Aerosol-resistant pipette tips	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
Consumables for the 7500 Fast Real-Time PCR System	
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 20 plates, 0.2-mL well; for use with 7300, 7500, and 7900HT Fast Real-Time PCR Systems	4306737[1]
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL, 20 plates; for use with 7500 Fast Real-Time PCR System	4346906
MicroAmp™ Optical 96-Well Reaction Plate with Barcode & Optical Adhesive Films, 100 plates with covers; for use with 7300 and 7500 Fast Real-Time PCR Systems	4314320
MicroAmp™ Optical 8-Cap Strips, 300 strips	4323032
MicroAmp™ Optical Adhesive Film Kit, 20 covers, 1 applicator, 1 optical cover compression pad	4313663
MicroAmp™ Optical Adhesive Film	4360954

[1] Not recommended for use with the 7500 Fast system. For 7500 Fast system reactions, use Cat. No. 4346906.

Create a plate document in the AccuSEQ™ software

1. In the home screen, select **Create Custom Experiment**.
2. In the **Experiment name** field, enter a unique name for the experiment.
3. Specify experiment information.
   a. Select experiment type **Quantitation – Standard Curve**.
   b. Select reagents **TaqMan™ Reagents**.
   c. Select ramp speed **Standard**.
4. In the **Plate Setup** screen, select the **Define Targets and Samples** tab.
5. Specify target information.
   a. Click **Add New Target**.
   b. Enter a host cell name in the target name field.
   c. Select reporter **FAM™** and quencher **NFQ_MGB**.
   d. Select a color for this target.
6. Specify IPC target information.
   a. Click **Add New Target**.
   b. Enter **IPC** in the target name field.
   c. Select reporter **VIC™** and quencher **NFQ_MGB**.
   d. Select a color for this target.
7. Define new samples.
   a. Click **Add New Sample**.
   b. In **Sample Name**, add the names of the samples you want to define.
   c. Click **Next**, or select the **Assign Targets and Samples** tab.
8. In the **Assign Targets and Samples** tab, define new targets.
   a. Follow the instructions in the top of the tab to set up the standards, unknowns, and negative controls.
   b. Click **Define and Set Up Standards** to open the **Define and Set Up Standards** dialog box to enter the appropriate settings and define the standard curve. When defined, click **Apply** and **Close**. The new standard curve is applied to the plate layout screen.
9. Assign the IPC to the standard curve wells.

10. In the **Run Method** screen, in the **Graphical View** tab.
    a. In **Reaction Volume Per Well**, enter **30 µL**
    b. Right-click the left column named **Holding Stage 1** and select **Delete Selected**. This 50°C hold stage is not needed.
11. Click the **Analysis** button in the left panel. In the **Analysis Settings** window on the right, set the default settings.
    a. On the **Ct Settings** tab, click **Edit Default Settings**. Then set **Threshold** to 0.2, set to **Automatic Baseline**, and then click **Save Changes**.

       Note: For CHO samples, a manual baseline of 3–12 is more appropriate.

    b. Select (highlight) both targets.
    c. In the right-hand window, select **Use Default Settings**.
    d. Click **Apply Analysis Settings**.

    Note: You can analyze the assays using Automatic or Manual Baseline, use the setting that yields the best standard curve. For CHO, the upper limit threshold for manual baseline analysis is 12.
12. Select **File ▸ Save as**, confirm that the file is named "resDNA_Template", then select **Save as a template file** in the drop-down list and close the template plate document.

    Note: You can reuse the plate template document to run an assay by opening the template file and choosing **Save As** to save the file with the experiment name.

Run the plate

1. In the toolbar, select **File ▸ Open**, navigate to the **resDNA_Template** file (created in "Create a plate document in the AccuSEQ™ software" on page 25 above), then click **Open**.
2. In the **Experiment Name** field, enter the appropriate experiment name, then click **Finish**.
3. Make any necessary changes to the test sample labels.
   - **Sample Volume**—not applicable; leave as default (0).
   - **Spike Volume**—volume of DNA added to the PCR (set to 10).
   - **Spike Standard Concentration**—expected spike amount per reaction (for example, 10pg).
   - **Reference**—the non-spiked sample; the mean quantity of reference is subtracted during % recovery calculation.
   - **Spike Input**—automatically calculated (double check if correct).

     Note: If incorrect, be sure **Spike Volume** is set to 10 and **Spike Standard Concentration** is the expected pg spike per PCR reaction.

4. Select **Save As** to save the new experiment as an EDS experiment file with the same name as entered in the **Experiment Name** field.
5. Load the plate into the instrument.
6. Click **Start Run**.
7. Select a run screen (**Amplification** plot, **Temperature** plot, or **Run method**) to monitor the progress of the run.

Analyze the results

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

1. In the toolbar, select **Analysis** ▸ **Analysis Settings**.
2. Click  (**Analyze**).
3. Select **Analysis** ▸ **QC Summary** in the left panel of the screen. Review the flag summary.
4. In the left panel, select **Analysis** ▸ **Standard Curve**. Verify the values for the Slope, Y-Intercept, R2, and Efficiency.
5. Select **File** ▸ **Print Report** to generate a hardcopy of the experiment, or click **Print Preview** to view and save the report as a *.pdf or *.html file.
6. Optional: Select **File** ▸ **Export**. In the **Export Data** menu, select file type ***.xls**. Click **Start Export**.

C

Use the kit with 7500 System SDS Software v1.5.1

Create the plate document, run the plate, and analyze the results with 7500 Fast SDS software

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.

Create a plate document

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

Plate document: setttings

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	resDNASEQ™ kit target cell lines	FAM™ (Select **None** for quencher)
	IPC	VIC™ (Select **None** for quencher)
PCR	Hold	Temp: **95**°C Time: **10:00**
	Cycling (Standard Mode)	Cycles: **40** Temp: **95**°C Time: **0:15** Temp: **60**°C Time: **1:00**
Analysis	CHO, *E. coli*, HEK293, Human, Vero, *Pichia*, NS0, and MDCK	**Automatic Baseline** *or* **Manual Baseline**[1] Threshold: **0.2** **Note:** For CHO, the upper limit for manual baseline analysis is 12.

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

Plate document: procedure

In the SDS software:

1. In the template **Assay** drop-down list, select **Absolute Quantification**.
2. In the **Run Mode** drop-down list, select **Standard 7500**.
3. Enter **resDNA_*Template*** in the **Plate** name field, then click **Next**.
4. Click **New Detector**:
   a. Enter the name of the target cell line in the **Name** field.
   b. Select **FAM™** in the **Reporter Dye** drop-down list.
   c. Select **(none)** in the **Quencher Dye** drop-down list.
   d. Select a color for the detector, then click **Create Another**.
5. Click **New Detector**:
   a. Enter **IPC** in the **Name** field.
   b. Select **VIC™** in the **Reporter Dye** drop-down list.
   c. Select **(none)** in the **Quencher Dye** drop-down list.
   d. Select a color for the detector, then click **OK**.
   e. Select the detectors, then click **Add>>** to add the detectors to the document (plate).
6. Select **ROX™** 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:

										Standard Curve (pg)		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	TS1	TS1	TS1		TS1 ERC	TS1 ERC	TS1 ERC			NTC	NTC	NTC
B	TS2	TS2	TS2		TS2 ERC	TS2 ERC	TS2 ERC					
C	TS3	TS3	TS3		TS3 ERC	TS3 ERC	TS3 ERC			0.03 pg	0.03 pg	0.03 pg
D										0.3 pg	0.3 pg	0.3 pg
E										3 pg	3 pg	3 pg
F	NEG	NEG	NEG							30 pg	30 pg	30 pg

										Standard Curve (pg)		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
G										300 pg	300 pg	300 pg
H										3,000 pg	3,000 pg	3,000 pg

8. Set tasks for each sample type by clicking on the **Task Column** drop-down list:
   a. NTC: target cell line detector task = **NTC**
   b. NEG, test samples, and ERC wells: target DNA detector task = **Unknown**
   c. IPC = **Unknown** for all wells
9. Set up the standard curve:
   a. Select the wells.
   b. Assign the tasks (target DNA = **Standard**) and enter the appropriate Quantity for each set of triplicates.

Tube label	Row-wells	Task	Quantity	Label (pg)
SD 1	H-10, 11, 12	Standard	3,000	3,000 pg
SD 2	G-10, 11, 12	Standard	300	300 pg
SD 3	F-10, 11, 12	Standard	30	30 pg
SD 4	E-10, 11, 12	Standard	3	3 pg
SD 5	D-10, 11, 12	Standard	0.3	0.3 pg
SD 6 (for CHO, Vero, MDCK, and NS0 only)	C-10, 11, 12	Standard	0.03	0.03 pg

10. Select the **Instrument** tab, then set thermal-cycling conditions:
    - Set the **thermal cycling reaction volume** to **30 µL**.
    - Set the reaction to **Standard Mode**.
    - Set the temperature and the time as shown in the following table:

Step	AmpliTaq Gold™ enzyme activation	PCR	
	Hold	Cycle (**40** Cycles)	
		Denature	Anneal/extend
Temp (°C)	95	95	60
Time (mm:sec)	10:00	0:15	1:00

    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**:
    a. Select **Manual Ct**.

b. In **Threshold**, enter **0.2**.

c. 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. For CHO, the upper limit threshold for manual baseline analysis is 12.

12. Select **File ▸ Save as**, confirm that the file is named "resDNA_Template", then select **SDS Templates (*.sdt)** in the **Save as type** drop-down 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.

Run the plate

1. In the SDS software, select **File ▸ New**, navigate to the **resDNA_Template** file (created in "Plate document: procedure" on page 29), 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.

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**.

D Good laboratory practices

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 (Kwok and Higuchi, 1989).

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.

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 decontamination solution.

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.

Note: Refer to “Prepare the PCR plate“ on page 15 for best practice.

Safety

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.

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. To obtain SDSs, see the "Documentation and Support" section in this document.
- 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! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication number	Description
resDNASEQ™ Quantitative DNA Kits Quick Reference	4469837	For brief instructions on using the resDNASEQ™ Quantitative DNA Kits.
PrepSEQ™ Residual DNA Sample Preparation Kit User Guide	4469838	For information on preparing samples for extraction.
PrepSEQ™ Residual DNA Sample Preparation Kit Quick Reference	4469839	For brief instructions on preparing samples for extraction.
AccuSEQ™ Real-Time PCR Software v3.0 User Guide	100084348	For information on AccuSEQ™ Real-Time PCR Software v3.0 with the QuantStudio™ 5 Real-Time PCR System
AccuSEQ™ Real-Time PCR Software v3.0 Quick Reference	100084353	For basic information on AccuSEQ™ Real-Time PCR Software v3.0 with the QuantStudio™ 5 Real-Time PCR System
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve	4347825	For information on the 7500 Fast instrument.
AccuSEQ™ software: Custom Quick Reference Card	4425585	For information on AccuSEQ™ software with the 7500 Fast instrument.

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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

References

Kwok, S., and Higuchi, R. 1989. Avoiding false positives with PCR. *Nature* 339:237–238.