USER GUIDE

applied biosystems® by life technologies™

resDNASEQ® Quantitative DNA Kits

resDNASEQ® Quantitative CHO DNA kit

resDNASEQ® Quantitative *E. coli* 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

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About This Guide

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

Revision history

Revision	Date	Description
B	January 2013	Add the following kits: • resDNASEQ® Quantitative CHO DNA Kit (Cat. no. 4402085) • resDNASEQ® Quantitative *E. coli* DNA Kit (Cat. no. • 4458435) • resDNASEQ® Quantitative Vero DNA Kit (Cat. no. • 4458444)
C	December 2014	Add the resDNASEQ® Quantitative Human DNA Kit (Cat. no. A26366)

Purpose

This guide provides step-by-step instructions for using the resDNASEQ® Quantitative DNA Kits to quantitate host-cell line residual DNA from CHO, *E. coli*, Human, Vero, *Pichia*, NS0, and MDCK cell lines.

Prerequisites

This guide uses conventions and terminology that assume a working knowledge of the Microsoft® Windows® operating system, the Internet, and Internet-based browsers.

1 resDNASEQ® Quantitative DNA Kits

Product description

The resDNASEQ® Quantitative DNA Kits are used to quantitate host-cell line residual DNA from CHO, *E. coli*, 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, refer to 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 3 ng to 30 fg per reaction) and the *E. coli*, Human, and *Pichia* assays require five dilutions (3 ng to 300 fg 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.

Kit contents and storage

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

The components for each of the resDNASEQ® Quantitative DNA Kits are shown in the tables below.

resDNASEQ® Quantitative CHO DNA Kit (Cat. no. 4402085)			
Cat. no.	**Reagent**	**Description**	**Storage**
4403965	**resDNASEQ® CHO DNA Control**		
4403967	CHO DNA Control	1 tube, 40 µL, 30ng/µL	Store at –15 to –25°C.
4405587	DNA Dilution Buffer (DDB)	1 bottle, 7 mL	Store at –15 to –25°C before first use. Store at 2 to 8°C after first use.
4402431	**resDNASEQ® CHO Real-Time PCR Reagents**		
4401975	2X Environmental Master Mix	2 tubes, 0.75 mL/tube	Store at –15 to –25°C before first use, protected from light. Store at 2 to 8°C after first use, protected from light.
4402087	10X CHO DNA Real-Time PCR Assay Mix	1 tube, 300 µL	Store at –15 to –25°C, protected from light.
362250	Negative Control (water)	1 tube, 1.0 mL	Store at –15 to –25°C before first use. Store at 2 to 8°C after first use.

resDNASEQ® Quantitative *E. coli* DNA Kit (Cat. no. 4458435)			
Cat. no.	**Reagent**	**Description**	**Storage**
4460973	**Box 1, SEQ Real-Time Core PCR Kit**		
4401975	2X Environmental Master Mix	2 tubes, 0.75 mL/tube	Store at –15 to –20°C before first use, protected from light. Store at 2 to 8°C after first use, protected from light.
362250	Negative Control (water)	1 tube, 1.0 mL	Store at –15 to –20°C before first use. Store at 2 to 8°C after first use.
4458447	**Box 2, resDNASEQ® *E. coli* Assay Mix**		
4458456	10X *E. coli* DNA Real-Time PCR Assay Mix	1 tube, 300 µL	Store at –15 to –25 °C, protected from light.
4458438	**Box 3, resDNASEQ® *E. coli* DNA Control**		
4458450	*E. coli* DNA Control	1 tube, 40 µL, 30ng/µL	Store at –15 to –20°C.
4405587	DNA Dilution Buffer (DDB)	1 bottle, 7 mL	Store at –15 to –20°C before first use. Store at 2 to 8°C after first use.

resDNASEQ® Quantitative Human DNA Kit (Cat. no. A26366)			
Cat. no.	**Reagent**	**Description**	**Storage**
100027323	**resDNASEQ® Human DNA Control**		
100027325	Human DNA Control	1 tube, 40 µL, 30 ng/µL	Store at –15 to –25°C.
4405587	DNA Dilution Buffer (DDB)	1 bottle, 7 mL	Store at –15 to –25°C before first use. Store at 2 to 8°C after first use.
100027320	**resDNASEQ® Human Real-Time PCR Reagents**		
4401975	2X Environmental Master Mix	2 tubes, 0.75 mL/tube	Store at –15 to –25°C before first use, protected from light. Store at 2 to 8°C after first use, protected from light.
100027324	10X Human DNA Real-Time PCR Assay Mix	1 tube, 300 µL	Store at –15 to –25°C, protected from light.
362250	Negative Control (water)	1 tube, 1.0 mL	Store at –15 to –25°C before first use. Store at 2 to 8°C after first use.

resDNASEQ® Quantitative Vero DNA Kit (Cat. no. 4458444)			
Cat. no.	**Reagent**	**Description**	**Storage**
4460973	**Box 1, SEQ Real-Time Core PCR Kit**		
4401975	2**X** Environmental Master Mix	2 tubes, 0.75 mL/tube	Store at –15 to –20°C before first use, protected from light. Store at 2 to 8°C after first use, protected from light.
362250	Negative Control (water)	1 tube, 1.0 mL	Store at –15 to –20°C before first use. Store at 2 to 8°C after first use.
4458446	**Box 2, resDNASEQ® Vero Assay Mix**		
4458461	10X Vero DNA Real-Time PCR Assay Mix	1 tube, 300 µL	Store at –15 to –20°C, protected from light.
4458463	**Box 3, resDNASEQ® Vero DNA Control**		
4458457	Vero DNA Control	1 tube, 40 µL, 30ng/µL	Store at –15 to –20°C.
4405587	DNA Dilution Buffer (DDB)	1 bottle, 7 mL	Store at –15 to –20°C before first use. Store at 2 to 8°C after first use.

resDNASEQ® Quantitative *Pichia* DNA Kit (Cat. no. 4464336)			
Cat. no.	**Reagent**	**Description**	**Storage**
4460973	**Box 1, SEQ Real-Time Core PCR Kit**		
4401975	2X Environmental Master Mix	2 tubes, 0.75 mL/tube	Store at –15 to –20°C before first use, protected from light. Store at 2 to 8°C after first use, protected from light.
362250	Negative Control (water)	1 tube, 1.0 mL	Store at –15 to –20°C before first use. Store at 2 to 8°C after first use.
4464334	**Box 2, resDNASEQ® *Pichia* Assay Mix**		
4464330	10X *Pichia* DNA Real-Time PCR Assay Mix	1 tube, 300 µL	Store at –15 to –25 °C, protected from light.
4464332	**Box 3, resDNASEQ® *Pichia* DNA Control**		
4464328	*Pichia* DNA Control	1 tube, 40 µL, 30ng/µL	Store at –15 to –20°C.
4405587	DNA Dilution Buffer (DDB)	1 bottle, 7 mL	Store at –15 to –20°C before first use. Store at 2 to 8°C after first use.

resDNASEQ® Quantitative NS0 DNA Kit (Cat. no. 4458441)			
Cat. no.	**Reagent**	**Description**	**Storage**
4460973	**Box 1, SEQ Real-Time Core PCR Kit**		
4401975	2X Environmental Master Mix	2 tubes, 0.75 mL/tube	Store at –15 to –20°C before first use, protected from light. Store at 2 to 8°C after first use, protected from light.
362250	Negative Control (water)	1 tube, 1.0 mL	Store at –15 to –20°C before first use. Store at 2 to 8°C after first use.
4458436	**Box 2, resDNASEQ® NS0 Assay Mix**		
4458445	10X NS0 DNA Real-Time PCR Assay Mix	1 tube, 300 µL	Store at –15 to –25 °C, protected from light.
4458453	**Box 3, resDNASEQ® NS0 DNA Control**		
4458443	NS0 DNA Control	1 tube, 40 µL, 30ng/µL	Store at –15 to –20°C.
4405587	DNA Dilution Buffer (DDB)	1 bottle, 7 mL	Store at –15 to –20°C before first use. Store at 2 to 8°C after first use.

resDNASEQ® Quantitative MDCK DNA Kit (Cat. no. 4464335)			
Cat. no.	**Reagent**	**Description**	**Storage**
4460973	**Box 1, SEQ Real-Time Core PCR Kit**		
4401975	2X Environmental Master Mix	2 tubes, 0.75 mL/tube	Store at –15 to –20°C before first use, protected from light. Store at 2 to 8°C after first use, protected from light.
362250	Negative Control	1 tube, 1.0 mL	Store at –15 to –20°C before first use. Store at 2 to 8°C after first use.
4464333	**Box 2, resDNASEQ® MDCK Assay Mix**		
4464329	10X MDCK DNA Real-Time PCR Assay Mix	1 tube, 300 µL	Store at –15 to –25 °C, protected from light.
4464331	**Box 3, resDNASEQ® MDCK DNA Control**		
4464327	MDCK DNA Control	1 tube, 40 µL, 30ng/µL	Store at –15 to –20°C.
4405587	DNA Dilution Buffer (DDB)	1 bottle, 7 mL	Store at –15 to –20°C before first use. Store at 2 to 8°C after first use.

Required materials not included in the kits

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

Instruments and software	Cat. no.
Applied Biosystems® 7500 Real-Time PCR System and applicable Applied Biosystems® 7500 System SDS Software	4351105
Applied Biosystems® 7500 Fast Real-Time PCR System and: • applicable Applied Biosystems® 7500 Fast System SDS Software *or* • AccuSEQ® Real-Time PCR Detection Software	4351107

Plates and plate consumables	Cat. no.
MicroAmp® Fast 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® Optical 96-Well Reaction Plate with Barcode, 20 plates for use with Applied Biosystems® 7500 Real-Time PCR System	4306737
MicroAmp® 96- & 384-Well Optical Adhesive Film, 100 covers	4311971
MicroAmp® Optical Adhesive Film Kit, 25 covers	4360954
MicroAmp® Adhesive Film Applicator, 5 applicators	4333183

Miscellaneous items	Source or Cat. no.
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-free Microfuge Tubes, 1.5-mL (1 box; 250 tubes/box)	AM12450

resDNASEQ® kit workflows

Workflow to prepare the serial dilutions and the standard curve samples

Prepare the control DNA serial dilutions for the standard curve (page 13)

↓

Prepare the reaction master mix (page 14)

↓

Prepare standard curve (page 15)

↓

Workflow to create the plate document, run the plate, and analyze the results

You can create the plate document, run the plate, and analyze the results using one of these two software applications:

Applied Biosystems® 7500 System SDS Software

or

AccuSEQ® Real-Time PCR Detection Software

Applied Biosystems® 7500 System SDS Software	AccuSEQ® Real-Time PCR Detection Software
Create a plate document (page 16)	Create a plate document (page 20)
↓	↓
Run the plate (page 19)	Run the plate (page 20)
↓	↓
Analyze the results (page 19)	Analyze the results (page 20)

Prepare control DNA serial dilutions for the standard curve

Prepare serial dilutions of control DNA to create a standard curve and to determine sample recovery rate. For CHO, Vero, MDCK, and NS0, prepare six serial dilutions (3 ng/reaction to 30 fg/reaction). For *E. coli*, Human, and *Pichia*, prepare five serial dilutions (3 ng/reaction to 300 fg/reaction).

Prepare the control DNA serial dilutions for the standard curve

1. Label seven nonstick 1.5-mL microfuge tubes: **NTC, SD1**, **SD2**, **SD3**, **SD4**, **SD5**, **and SD6**.
2. Add 33 µL of DNA dilution buffer (DDB) to tube NTC. Put aside.
3. Add 990 µL of DDB to tube SD1.
4. Add 450 µL of 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 quick-spin.
7. Perform the serial dilutions:
   a. Add 10 µL of the DNA control to the tube that is labeled SD1, then vortex thoroughly and quick-spin.

b. Transfer 50 µL of the DNA from tube SD1 to tube SD2, then vortex thoroughly and quick-spin.

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*, Human, and *Pichia*) or SD6 (CHO, Vero, MDCK, and NS0). Final dilutions are shown in the table. After each transfer, vortex thoroughly.

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	3000 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 at 4°C for use on the day of preparation. Otherwise, store the tubes at –20°C and use within 1 week.

Prepare the reaction master mix

1. Determine the number of controls and test samples whose DNA content you will quantify.
2. Thaw all kit reagents completely at room temperature, thoroughly mix reagents and spin down contents.
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.

   IMPORTANT! Environmental Master Mix must be 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 primer/probe mix	3 µL	**118.8 µL**
2X Environmental Master Mix	15 µL	**594 µL**

Kit reagents	Volume for 1 30-µL reaction	Volume for 36 30-µL reactions (includes 10% overage)
DNA template	10 µL	Add DNA template to each well separately, not as part of Master Mix
Total	30 µL	**792 µL**

Prepare standard curve

When preparing the standards, note these guidelines:

- DNA quantification standards are critical for accurate analysis of run data.
- Mistakes or inaccuracies in making the dilutions directly affect the quality of results.
- The quality of pipettes and tips and the care used in measuring and mixing dilutions affect accuracy.

Note: If you previously prepared and stored Standard Curve dilutions, make sure they are fully thawed. Vortex and quick-spin before use.

1. Label 6 standard tubes with **SC 1** to **SC 6** as shown in the first column of the table below. (You prepared the NTC tube in step 2 on page 13.)

Standard curve (SC) tube	Volume of PCR reaction mix	Volume to transfer from indicated serial dilution (SD) tube	Amount of DNA in the PCR well
SC 1	66 µL	(SD 1) 33 µL	3000 pg
SC 2	66 µL	(SD 2) 33 µL	300 pg
SC 3	66 µL	(SD 3) 33 µL	30 pg
SC 4	66 µL	(SD 4) 33 µL	3 pg
SC 5	66 µL	(SD 5) 33 µL	0.3 pg
SC 6 (CHO, Vero, MDCK, and NS0 only)	66 µL	(SD 6) 33 µL	0.03 pg
NTC	66 µL	33 µL DDB (DNA Dilution Buffer)	0.0 pg

2. Add 66 µL of the PCR reaction mix (Environmental Master Mix) to each tube.
3. Add 33 µL from SD tubes with the corresponding number designation (as shown in the table) to each SC tube.
4. Vortex each tube briefly and quick-spin. Dispense 30 µL from each tube into three replicate wells. Pipet the reagents for the standard curve starting with the lowest concentration and finishing with highest concentration.

Prepare the test samples

1. Set up a 96-well PCR reaction plate using the example plate layout shown below (36 reactions for standard curve and 3 test samples), where:
   - NTC = no template control.
   - NEG = negative extraction control.
   - TS = test sample.
   - ERC = extraction recovery control

										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			SC6	SC6	SC6
D										SC5	SC5	SC5
E										SC4	SC4	SC4
F	NEG	NEG	NEG							SC3	SC3	SC3
G										SC2	SC2	SC2
H										SC1	SC1	SC1

Note: The plate layout is a suggested plate layout. Adjust the layout according to the number of test samples to be run.

2. Add 20 µL PCR Master mix to each sample well.
3. Add 10 µL each of extracted sample DNA to the appropriate wells.
4. Seal the plate with an optical film, vortex the plate, and then quick-spin with a mini plate centrifuge that is compatible with 96-well plates.

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 are using the AccuSEQ® software, see "Create the plate document, run the plate, and analyze the results with AccuSEQ® Real-Time PCR Detection Software" on page 20. 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).

If you have run the assay frequently, you can use the table below to enter settings in Plate Document fields. New users should follow the detailed instructions that appear immediately after the table.

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*, Human, Vero, *Pichia*, NS0, and MDCK	**Automatic Baseline** *or* **Manual Baseline**† Threshold: **0.2**

† You can analyze the assays using Automatic or Manual Baseline, whichever results in the best standard curve.

Detailed instructions

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**.
6. Select **ROX** as the passive reference dye.

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**
G										**300 pg**	**300 pg**	**300 pg**
H										**3000 pg**	**3000 pg**	**3000 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 as shown in the following table:
   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)
SC 1	H-10, 11, 12	Standard	3000	3000 pg
SC 2	G-10, 11, 12	Standard	300	300 pg
SC 3	F-10, 11, 12	Standard	30	30 pg
SC 4	E-10, 11, 12	Standard	3	3 pg
SC 5	D-10, 11, 12	Standard	0.3	0.3 pg
SC 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**.
    - For the 7500 Fast system, 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

Step	AmpliTaq Gold® enzyme activation	PCR	
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. 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.

Run the plate

1. In the SDS software, select **File ▸ New**, navigate to the **resDNA_Template** file (created in step 11 above), then click **Open**.
2. In the Plate Name field, enter **ResDNA_** ***date of Assay***, then click **Finish**.
3. Make any necessary changes to the test sample labels.
4. Load the plate on the instrument.
5. 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 for CHO, *E. coli*, Human, Vero, *Pichia*, NSO, and MDCK.

1. Select the **Results** tab, then select **Analysis ▸ Analysis Settings**.
2. In the Analysis Settings window, enter the following settings, then click **OK**:
   a. Select **Manual Ct**.
   b. In the Threshold field, enter **0.2**.
   c. Select **Automatic Baseline** or **Manual Baseline**.

   Note: You can analyze the assays using Automatic or Manual Baseline, whichever results in the best standard curve.
3. Click (**Analyze**) in the toolbar, then wait while the plate is analyzed.
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. Select **File ▸ Export ▸ Results**. In the Save as type drop-down list, select **Results Export Files (*.csv)**, then click **Save**.

Create the plate document, run the plate, and analyze the results with AccuSEQ® Real-Time PCR Detection Software

The following instructions apply only to the 7500 Fast instrument with AccuSEQ® Real-Time PCR Detection Software. If you are using the 7500 Fast instrument SDS software, see "Create the plate document, run the plate, and analyze the results with 7500 Fast SDS software" on page 16). If you use a different instrument or software, refer to the applicable instrument or software documentation.

Create a plate document

1. In the home screen, select **Create Custom Experiment**.
2. In the Experiment name field, enter **resDNA_*Template***.
3. Select experiment type **Quantitation – Standard Curve**. Select reagents **TaqMan Reagents** and ramp speed **Standard**.
4. In the Plate Setup screen, select the **Define Targets and Samples** tab.
5. Click **Add New Target**. Enter a name in the target name field. Select reporter **FAM** and quencher **NFQ_MGB**. Select a color for this target.
6. Click **Add New Target**. Enter **IPC** in the target name field. Select reporter **VIC** and quencher **NFQ_MGB**. Select a color for this target.
7. Click **Add New Sample**.
8. Click **Define and Set up Standards**.
9. In the **Assign Targets and Samples** tab, for each sample, click the sample, then click the appropriate well to assign the sample and target to the well.
10. In the Run Method screen, set the Reaction Volume Per Well to **30 µL**.
11. 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 whenever you run the assay.

Run the plate

1. In the toolbar, select **File ▸ Open**, navigate to the **resDNA_Template** file (created in step 11 above), then click **Open**.
2. In the Plate Name field, enter **ResDNA_ *date of Assay***, then click **Finish**.
3. Make any necessary changes to the test sample labels.
4. Load the plate into the instrument.
5. Click **Start Run**.
6. 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 CHO, *E. coli*, Human, Vero, *Pichia*, NSO, and MDCK.

1. In the toolbar, select **Analysis ▸ Analysis Settings**.

2. In the Analysis Settings window, enter the following settings, then click **OK**:
   a. Deselect **Automatic Threshold**.
   b. In the Threshold field, enter **0.2**.
   c. Select **Automatic Baseline** or **Manual Baseline**.

   Note: You can analyze the assays using Automatic or Manual Baseline, whichever results in the best standard curve.
3. Click 

(**Analyze**).
4. Select **Analysis** ▸ **QC Summary** in the left panel of the screen. Review the flag summary.
5. In the left panel, select **Analysis** ▸ **Standard Curve**. Verify the values for the Slope, Intercept, R2, and Efficiency.
6. In the Standard Curve screen, click  (**Save as .jpg image**) to save the plot of the standard curve.
7. Select **File** ▸ **Export**. In the Export Data menu, select file type ***.xls**. Click **Start Export**.
8. 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.

Troubleshooting

Observation	Possible cause	Action
Slope for the standard curve is outside the typical range, or R_2 value is significantly less than 0.98	When applying detectors for standards, the Task and Quantity were applied to the wrong detector. *or* The incorrect Quantity was entered.	1. 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.
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* The compression pad was not used during the run.	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 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 ΔR_n 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.

A Good Laboratory Practices

Residual DNA quantification

Diluting test samples for residual DNA sample preparation

Test samples from early in the purification process often contain levels of DNA that are above the highest point on the standard curve of the residual DNA assay. Dilute the samples appropriately (from 1:100 up to 1:10,000) prior to sample preparation.

The PrepSEQ® sample preparation protocol is optimized for highly efficient recovery of DNA from complex mixtures of proteins, buffer and salts. Because of this, recovery of DNA from samples diluted in water or TE is often not efficient. We recommend:

- 50 mM Tris, pH 8.0, 0.5 M NaCl

  or

- PBS

Preparing serial dilutions and a standard curve

Follow these guidelines when you prepare serial dilutions with the standard DNA provided in the kit to generate the standard curve for quantitation of DNA in test samples.

- Prepare dilutions in nonstick 1.5-mL microfuge tubes (Cat. no. AM12450).
- 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. Quick-spin to collect all the liquid at the bottom before making next dilution.
- Use DNA Dilution Buffer (DDB) for all dilutions of Standard DNA.
- Label the top of each tube as indicated in the protocol.
- You can store the standard curve preparation at 2° — 8°C for up to 1 week and use it for multiple assays.

Preventing PCR contamination

PCR assays require special laboratory practices to avoid false positive amplifications. The efficiency and high sensitivity of these assays can lead to amplification of a single DNA molecule.

When preparing samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) when preparing samples for PCR amplification.
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate work areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes and reaction plates carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Clean lab benches and equipment after use with freshly diluted 10% bleach solution.

Avoiding false positives due to cross-contamination

To avoid false positives due to cross-contamination:

- Prepare and close all negative control and unknown sample tubes before pipetting the positive control.
- Do not open tubes after amplification.
- Use different sets of pipettors when pipetting negative control, unknown, and positive control samples.

Plate layout suggestions

- For each plate row, dispense in sequence from left to right the: negative controls, unknown samples, and positive controls (at the end of the row or column).
- Place positive controls in one of the outer columns.
- If possible, separate all samples from each other by at least one well; if space is limiting, place at least one well between unknown samples and controls.
- Be aware that caps come in strips of 8 or 12.

B

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, etc). 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, and 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 adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's 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 necessary) 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.

Specific chemical handling

CAS	Chemical	Phrase
26628-22-8	Sodium Azide	Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Biological hazard safety

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. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

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.

Documentation and Support

Related documentation

For information on new assays, go to: **www.microseq.com**

- For brief instructions on using the resDNASEQ® Quantitative DNA Kits, see the *resDNASEQ® Quantitative DNA Kits Quick Reference* (Pub. no. 4469837).
- For information on preparing samples for extraction, refer to the *PrepSEQ® Residual DNA Sample Preparation Kit User Guide* (Pub. no. 4469838).
- For brief instructions on preparing samples for extraction, refer to the *PrepSEQ® Residual DNA Sample Preparation Kit Quick Reference* (Pub. no. 4469839).
- For information on the 7500 Fast instrument, refer to the *Applied Biosystems® 7300/7500/7500 Fast Real-Time PCR System: Absolute Quantitation Using Standard Curve Getting Started Guide* (Pub. no. 4347825).
- For information on AccuSEQ® software with the 7500 Fast instrument, refer to the *Applied Biosystems® AccuSEQ® Real-Time PCR Detection Software Custom Experiments Quick Reference Card* (Pub. no. 4425585).

Portable document format (PDF) versions of this guide and the documents listed above are available at **www.lifetechnologies.com**

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents

- Obtain information about customer training
- Download software updates and patches

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 found on Life Technologies website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.