

# resDNASEQ™ Quantitative E1A DNA Fragment Length Kit

## USER GUIDE

for use with PrepSEQ™ Nucleic Acid Sample Preparation Kit

**Catalog Numbers** A51969 and A51970

**Publication Number** MAN0025643

**Revision** A.0



Life Technologies Ltd | 7 Kingsland Grange | Woolston, Warrington WA1 4SR | United Kingdom

For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

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

**DISCLAIMER:** TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

**Revision history:** Pub. No. MAN0025643

Revision	Date	Description
A.0	17 December 2021	New document for the launch of the resDNASEQ™ Quantitative E1A DNA Fragment Length Kit (Cat. No. <a href="#">A51969</a> ).

**Important Licensing Information:** These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

**TRADEMARKS:** All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license.

©2021 Thermo Fisher Scientific Inc. All rights reserved.

# Contents

■	<b>CHAPTER 1</b>	<b>Product information</b>	<b>5</b>
		Product description	5
		Contents	5
		Required materials not supplied	6
		Workflow	8
■	<b>CHAPTER 2</b>	<b>Methods</b>	<b>9</b>
		Prepare the E1A DNA control serial dilutions	9
		Guidelines for standard dilutions	9
		Prepare the control serial dilutions (short, medium, and long fragment)	9
		Prepare the Real-Time PCR Plate	10
		Prepare the PCR reaction mixes	10
		Prepare the PCR plate	12
		Setup, run, and analyze samples with AccuSEQ™ Software v3.1 on the QuantStudio™ 5 Real-Time PCR Instrument	13
		Sign in to the AccuSEQ™ Real-Time PCR Software	13
		Create an E1A resDNASEQ™ template	14
		Edit the run method and optical filter selection	17
		Assign plate and well attributes	18
		Load the plate in the instrument	20
		Start the run	21
		Monitor the run	21
		Review the results	23
■	<b>APPENDIX A</b>	<b>Troubleshooting</b>	<b>27</b>
■	<b>APPENDIX B</b>	<b>Use the kit with the 7500 Fast Real-Time PCR Instrument and AccuSEQ™ software v2.x</b>	<b>30</b>
		Required materials not supplied	30
		Plate document: settings	31
		Create a plate document in the AccuSEQ™ software	31
		Run the plate	33
		Analyze the results	34
		Determine the copy number of E1A fragments	35

- **APPENDIX C Use the kit with 7500 System SDS Software v1.5.1** ..... 36
  - Create the plate document, run the plate, and analyze the results with 7500 Fast SDS software ..... 36
    - Create a plate document ..... 36
    - Run the plate ..... 39
    - Analyze the results ..... 39
  
- **APPENDIX D Good laboratory practices** ..... 40
  - Work area setup and lab design ..... 40
  - Good laboratory practices for PCR and RT-PCR ..... 40
    - Avoiding false positives due to cross-contamination ..... 40
  - Good pipetting practices ..... 41
  
- **APPENDIX E Safety** ..... 42
  - Chemical safety ..... 42
  - Biological hazard safety ..... 43
  
- **APPENDIX F Documentation and support** ..... 44
  - Related documentation ..... 44
  - Customer and technical support ..... 44
  - Limited product warranty ..... 45



# Product information

---

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

---

## Product description

The resDNASEQ™ Quantitative E1A DNA Fragment Length Kit is used to measure and quantify residual host-cell DNA fragments of the E1A gene from HEK293 cell lines. HEK293 cell lines are predominantly used in the development of gene therapy, cell-based vaccines, and similar biopharmaceutical products. Use the kit to quantify extracted host-cell associated E1A DNA from test samples. For extraction information, see the *PrepSEQ™ Sample Preparation Kits User Guide* (Pub. No. 4469838).

The resDNASEQ™ Quantitative E1A DNA Fragment Length Kit uses TaqMan™ quantitative PCR to perform rapid, specific quantitation of residual host-cell E1A 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 curves used to quantify the DNA in test samples, the short, medium, and long fragment assays require five dilutions (1.00E+08 to 10 copies per reaction). Control DNA for standard curve generation is included in the kit. In addition, the kit uses an internal positive control (IPC) to evaluate the performance of each PCR reaction.

## Contents

Table 1 resDNASEQ™ Quantitative E1A DNA Fragment Length Kit (Cat. No. [A51969](#))

Contents	Amount	Storage
<b>resDNASEQ™ E1A Real-Time PCR Reagents</b>		
Environmental Master Mix 2.0	5.0 mL	–25°C to –15°C before first use, protect from light 2–8°C after first use, protect from light
E1A DNA Short Fragment Assay Mix	0.40 mL	–25°C to –15°C, protect from light
E1A DNA Medium Fragment Assay Mix	0.66 mL	
E1A DNA Long Fragment Assay Mix	0.40 mL	

Table 1 resDNASEQ Quantitative E1A DNA Fragment Length Kit (Cat. No. A51969) (continued)

Contents	Amount	Storage
Negative Control (Water)	3 × 1.0 mL	–25°C to –15°C before first use 2–8°C after first use
<b>resDNASEQ™ E1A DNA Control</b>		
E1A DNA Control, 6.30E+08 copies/μL	0.12 mL	–25°C to –15°C
DNA Dilution Buffer	7.0 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](http://thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](http://fisherscientific.com) or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
<b>Instrument</b>	
QuantStudio™ 5 Real-Time PCR System with AccuSEQ™ Real-Time PCR Software v3.1 or later	Contact your local sales representative.
<b>Consumables</b>	
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	<a href="#">4346906</a>
MicroAmp™ 96-Well Base	<a href="#">N8010531</a> , 10 bases
MicroAmp™ Optical Adhesive Film	<a href="#">4311971</a> , 100 covers <a href="#">4360954</a> , 25 covers
MicroAmp™ Adhesive Film Applicator	<a href="#">4333183</a> , 5 applicators
<b>Miscellaneous items</b>	
Vortex mixer	MLS
Microcentrifuge	MLS
Disposable gloves	MLS
Pipettes	MLS
Aerosol-resistant micropipette tips	MLS

(continued)

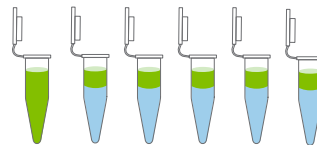
Item	Source
For the PCR plate: Fisher Scientific™ Mini Plate Spinner Centrifuge, 120- or 230-volt	14-100-143 (120-volt), 14-100-141 (230-volt)
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL (1 box; 250 tubes/box)	<a href="#">AM12450</a>

## Workflow

### E1A DNA Fragment Length Kit Workflow

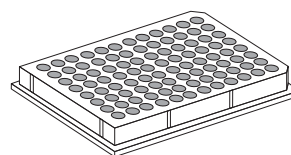
10 min

**Prepare the E1A DNA control serial dilutions**



5 min

**Prepare the PCR reaction mixes**



30 min

**Prepare the Real-Time PCR Plate**

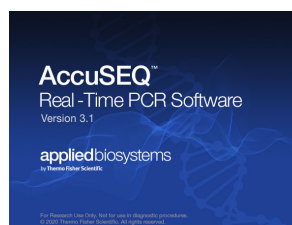
5 min

**Create an E1A resDNASEQ™ template**



2 hrs  
and  
30 mins

**Setup, run, and analyze samples with AccuSEQ™ Software v3.1 on the QuantStudio™ 5 Real-Time PCR Instrument**







## Prepare the E1A DNA control serial dilutions

### 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 for 15–30 seconds 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 serial dilutions (short, medium, and long fragment)

1. Label nonstick 1.5-mL microfuge tubes: **NTC**, **SD1**, **SD2**, **SD3**, **SD4**, **SD5**, **SD6** [used for limit of detection (LOD)].
2. Add DNA Dilution Buffer to tubes **SD1–SD6** according to Table 2.
3. Remove the tube of DNA control ( $6.3 \times 10^8$  copies/ $\mu\text{L}$ ) from the freezer. Thaw on ice.
4. After the DNA has thawed, vortex for 15–30 seconds, then briefly centrifuge for less than 5 seconds.
5. Perform the serial dilutions:  
The amounts are sufficient for 3 replicates per standard (short, medium, and long).
  - a. Add 10  $\mu\text{L}$  of the DNA control to the tube that is labeled SD1, vortex for 15–30 seconds, then briefly centrifuge for less than 5 seconds.
  - b. Transfer 2  $\mu\text{L}$  of the DNA from tube SD1 to tube SD2, vortex for 15–30 seconds, then briefly centrifuge for less than 5 seconds.
  - c. Transfer 2  $\mu\text{L}$  of the DNA from tube SD2 to tube SD3, vortex for 15–30 seconds, then briefly centrifuge for less than 5 seconds.
  - d. Transfer 20  $\mu\text{L}$  of the DNA from tube SD3 to tube SD4, vortex for 15–30 seconds, then briefly centrifuge for less than 5 seconds.
  - e. Transfer 6  $\mu\text{L}$  of the DNA from tube SD4 to tube SD5, vortex for 15–30 seconds, then briefly centrifuge for less than 5 seconds.

- f. Transfer 100  $\mu\text{L}$  of the DNA from tube SD5 to tube SD6, vortex for 15–30 seconds, then briefly centrifuge for less than 5 seconds.

**Table 2 Standard curve dilutions**

Serial dilution (SD) tube	Dilution	Total volume	Final copies/ PCR reaction (3 $\mu\text{L}$ )
SD 1	10 $\mu\text{L}$ DNA control + 179 $\mu\text{L}$ DDB	189 $\mu\text{L}$	1.00E+08
SD 2	2 $\mu\text{L}$ SD1 + 198 $\mu\text{L}$ DDB	200 $\mu\text{L}$	1.00E+06
SD 3	2 $\mu\text{L}$ SD2 + 198 $\mu\text{L}$ DDB	200 $\mu\text{L}$	10,000
SD 4	20 $\mu\text{L}$ SD3 + 180 $\mu\text{L}$ DDB	200 $\mu\text{L}$	1,000
SD 5	6 $\mu\text{L}$ SD4 + 194 $\mu\text{L}$ DDB	200 $\mu\text{L}$	30
SD 6 (LOD)	100 $\mu\text{L}$ SD5 + 200 $\mu\text{L}$ DDB	300 $\mu\text{L}$	10

**Note:** Adjust the calculated volumes according to the number of reactions required. The same standard curve material is used for all three assays (short, medium and long).

6. Store the DNA dilution tubes:

Temperature	For use
4°C	Same day
-20°C	$\leq 1$ week
-20°C	SD1 in single-use aliquots $\leq 6$ months

## Prepare the Real-Time PCR Plate

### Prepare the PCR reaction mixes

Three reaction mixes are made for short, medium, and long fragment sizes.

- Determine the number of reactions needed for the test sample dilutions that you will quantify.
- Thaw all kit reagents completely at room temperature, vortex the reagents for 15–30 seconds, then briefly centrifuge for less than 5 seconds.
- Prepare a PCR reaction mix using the reagents and volumes shown in the table below.
  - Multiply the PCR reaction volume for one reaction (30  $\mu\text{L}$ ) 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 per reaction (30 $\mu$ L)	Volume for 35 30- $\mu$ L reactions (includes 10% overage)
	Short and long assays	
TaqMan™ Environmental Master Mix 2.0	15.0 $\mu$ L	525.0 $\mu$ L
Assay mix (short or long)	3.6 $\mu$ L	126.0 $\mu$ L
Negative Control (Water)	8.4 $\mu$ L	294.0 $\mu$ L
DNA template	3 $\mu$ L	Add DNA template to each well separately, not as part of Master Mix
<b>Total</b>	<b>30 <math>\mu</math>L</b>	<b>27 <math>\mu</math>L per reaction</b>

Kit reagents	Volume per reaction (30 $\mu$ L)	Volume for 35 30- $\mu$ L reactions (includes 10% overage)
	Medium assay	
TaqMan™ Environmental Master Mix 2.0	15.0 $\mu$ L	525.0 $\mu$ L
Assay mix (medium)	6.0 $\mu$ L	210.0 $\mu$ L
Negative Control (Water)	6.0 $\mu$ L	210.0 $\mu$ L
DNA template	3 $\mu$ L	Add DNA template to each well separately, not as part of Master Mix
<b>Total</b>	<b>30 <math>\mu</math>L</b>	<b>27 <math>\mu</math>L per reaction</b>

## Prepare the PCR plate

Plate setup differs slightly for each AccuSEQ™ System. See your software user guide for specific instructions.

	Short			Medium			Long					
	1	2	3	4	5	6	7	8	9	10	11	12
A	SD1	SD1	SD1	SD1	SD1	SD1	SD1	SD1	SD1	SA1	SA1	SA1
B	SD2	SD2	SD2	SD2	SD2	SD2	SD2	SD2	SD2	SA1	SA1	SA1
C	SD3	SD3	SD3	SD3	SD3	SD3	SD3	SD3	SD3	SA1	SA1	SA1
D	SD4	SD4	SD4	SD4	SD4	SD4	SD4	SD4	SD4	SA2	SA2	SA2
E	SD5	SD5	SD5	SD5	SD5	SD5	SD5	SD5	SD5	SA2	SA2	SA2
F	SD6	SD6	SD6	SD6	SD6	SD6	SD6	SD6	SD6	SA2	SA2	SA2
G	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	SA3	SA3	SA3
H	ERC	ERC	ERC	ERC	ERC	ERC	SA3	SA3	SA3	SA3	SA3	SA3

**Figure 1 Recommended plate setup**

- SD—Standard dilutions
- SA—Sample
- NTC—No template control
- ERC—Extraction recovery control

**Note:** Thermo Fisher Scientific recommends testing several sample dilutions to determine a dilution that falls in the standard curve range prior to running the E1A assays.

1. Add 27  $\mu$ L of the appropriate PCR reaction mix to the respective assay wells as shown in the plate layout.
2. Add 3  $\mu$ L of PCR NTC to the appropriate wells.
3. Add 3  $\mu$ L each of extracted sample DNA to the appropriate wells.

**Note:**

- For fresh extracted samples, thoroughly vortex the tubes or deep well plates for 15 seconds before adding samples to the wells.
- For frozen and thawed samples, vortex thoroughly for 30 seconds, then centrifuge the samples to collect liquid to the bottom of tubes or plates.
- If you prepared samples with the automated protocol, use a multichannel pipette to transfer the extracted sample.

4. Add 3  $\mu$ L of standard dilutions to the appropriate wells.

**Note:**


- Vortex each standard dilution for 15–30 seconds before adding to the reaction mixes.
- Use different sets of pipettors to dispense test sample and standard curve dilutions to avoid cross-contamination of test samples.

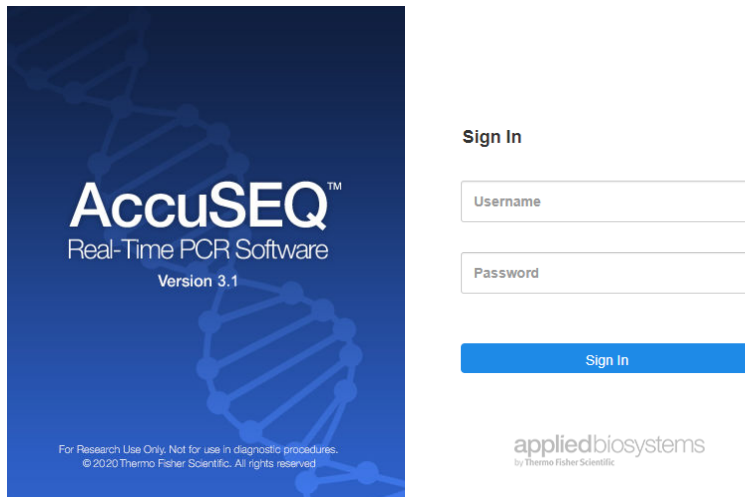
5. Use a film applicator to seal the plate with optical film. Vortex the plate for 10–15 seconds, then briefly centrifuge (less than 5 seconds) with a miniplate centrifuge that is compatible with 96-well plates.

# Setup, run, and analyze samples with AccuSEQ™ Software v3.1 on the QuantStudio™ 5 Real-Time PCR Instrument

## Sign in to the AccuSEQ™ Real-Time PCR Software

Thermo Fisher Scientific recommends configuring the Windows™ 10 screen save feature to require sign in when the screensaver is activated. This prevents other users from accessing the AccuSEQ™ Real-Time PCR Software and making changes.


1. Launch the AccuSEQ™ Real-Time PCR Software by double-clicking the AccuSEQ icon .
2. Enter the **Username**, then **Password**.  
(First login only) The default username is **Administrator** and the default password is **Administrator**.
3. Click **Sign in**.



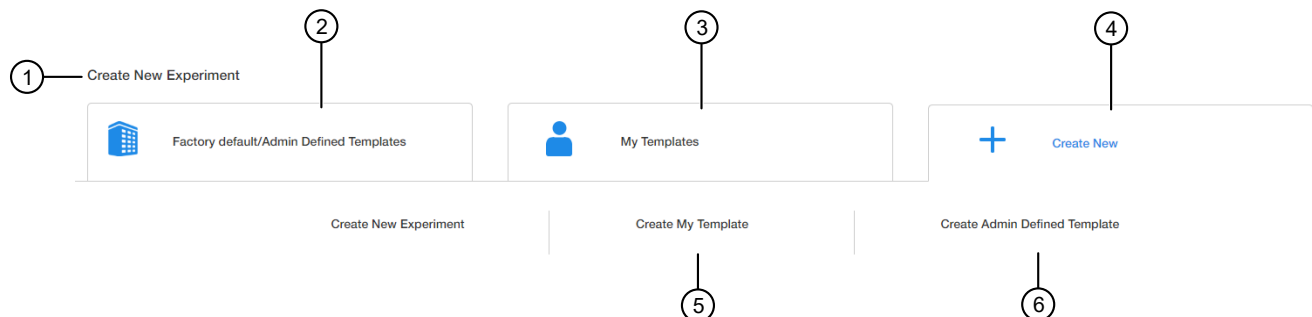
The following restrictions may be seen in the software:

- Access to functions in the software is based on the permissions associated with the individual user account.
- If a user account does not have permission to perform a function, the function is grayed out in the software.
- If the system is configured for password expiration, you will be periodically prompted to change your password. If the system is configured to monitor failed log in attempts, you will be locked out of the software if you incorrectly enter your user name or password more than a specified number of times.

## Create an E1A resDNASEQ™ template

Create a new template in the  (**Home**) screen of the AccuSEQ™ Real-Time PCR Software v3.1.

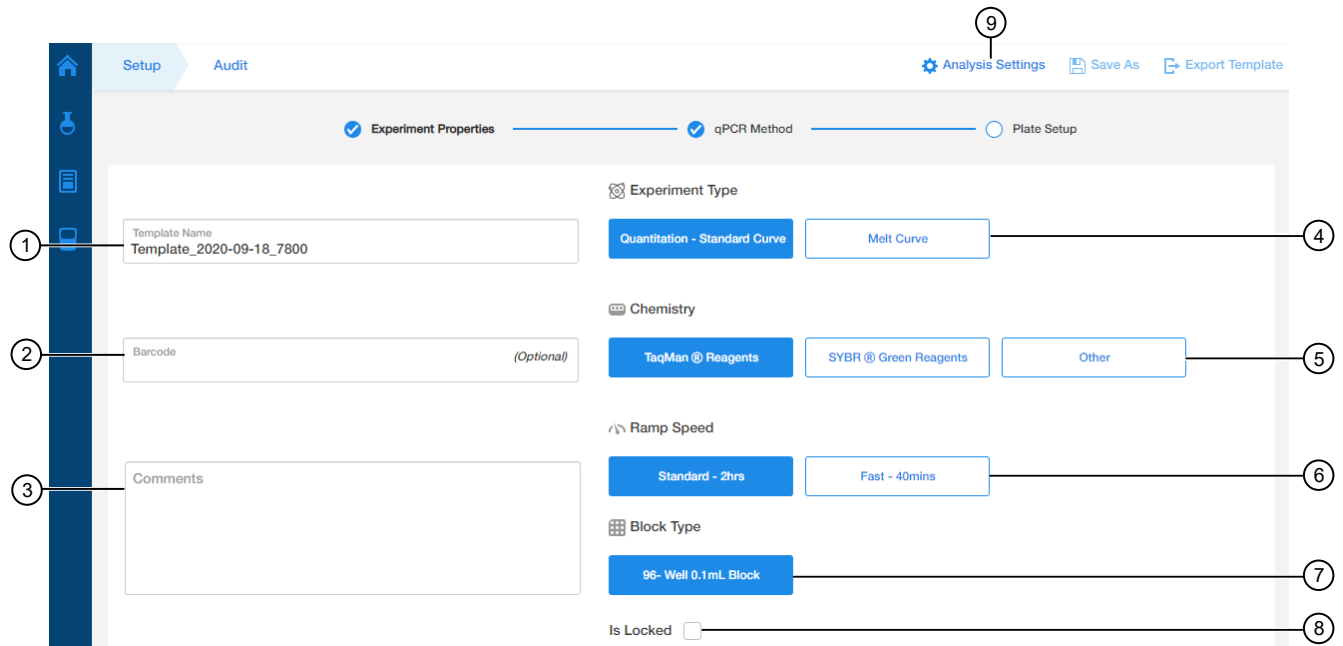
1. Click **+ Create New** on the home screen.



- ① **Create New Experiment** pane
- ② **Factory default/Admin Defined Templates**—List of existing default or Admin Defined templates. These templates can be used as templates for new experiments.
- ③ **My Templates**—List of templates available to the user that is signed in. These templates can be used as templates for new experiments.
- ④ **Create New**—Used to create an experiment or template with no pre-existing settings.
- ⑤ **Create My Template**—Used to create a new template (stored locally in **My Templates**).
- ⑥ **Create Admin Defined Template**—Used to create a new template (Administrator only).

2. Select **Create My Template** or **Create Admin Defined Template**.

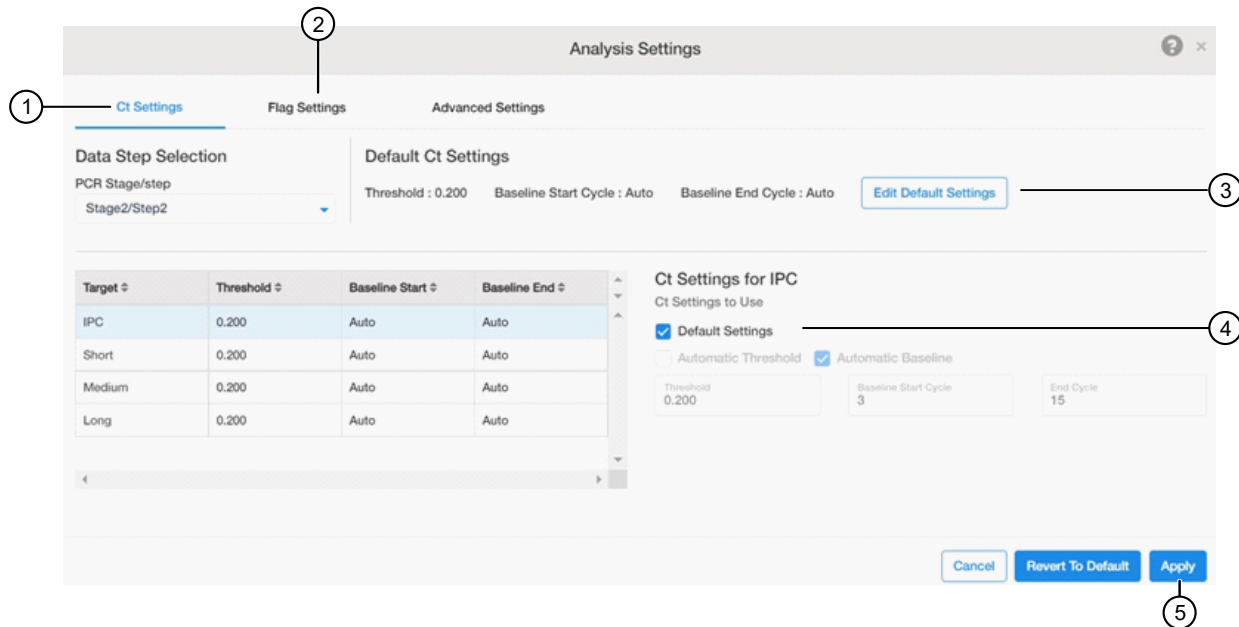
3. Edit the **Experiment Properties** as required.



- ① Software-generated **Template Name**
- ② **Barcode** field
- ③ **Comments** field
- ④ **Experiment Type**
- ⑤ **Chemistry**
- ⑥ **Ramp Speed**
- ⑦ **Block Type** (fixed as a 96-Well 0.1mL block).
- ⑧ **Is Locked** checkbox (Only Administrators can create locked **Admin Defined Templates**).
- ⑨ **Analysis Settings**

- a. In the **Template Name** field, modify the template name. For example, E1A template.
  - b. (Optional) Enter information in the **Comments** field.
  - c. In the **Setup** tab, select:
    - **Experiment Type—Quantitation-Standard Curve**
    - **Chemistry—TaqMan Reagents**
    - **Ramp Speed—Standard-2hrs**
    - **Block Type—96-Well 0.1mL Block**
  - d. (Optional) Select **Is Locked** to lock the template. If locked, users are unable to edit the template.
4. Click **Analysis settings** to change the default  $C_t$  Settings and Flag Settings.
- a. In the  **$C_t$  Settings** tab, click **Edit Default Settings**.
  - b. Deselect **Automatic Threshold**, then enter 0.200.
  - c. Ensure that **Automatic Baseline** is selected.
  - d. Click **Save Changes**.

e. Deselect **Default Settings**, then click **Apply** to save any changes before closing the window.



- ① **C<sub>t</sub> Settings**
- ② **Flag Settings**
- ③ **Edit Default Settings** button
- ④ **Default Settings** checkbox
- ⑤ **Apply** button

f. In the **Flag Settings** tab, deselect the following flags.

- **CQCONF**—Low C<sub>q</sub> confidence
- **EXPFAIL**—Exponential algorithm failed
- **NOAMP**—No amplification
- **NOSIGNAL**—No signal in well

---

**Note:** Use the scrollbar on the right to scroll down the list of flags.

---

g. Click **Apply** to save any changes before closing the window.

5. Click **Next**.

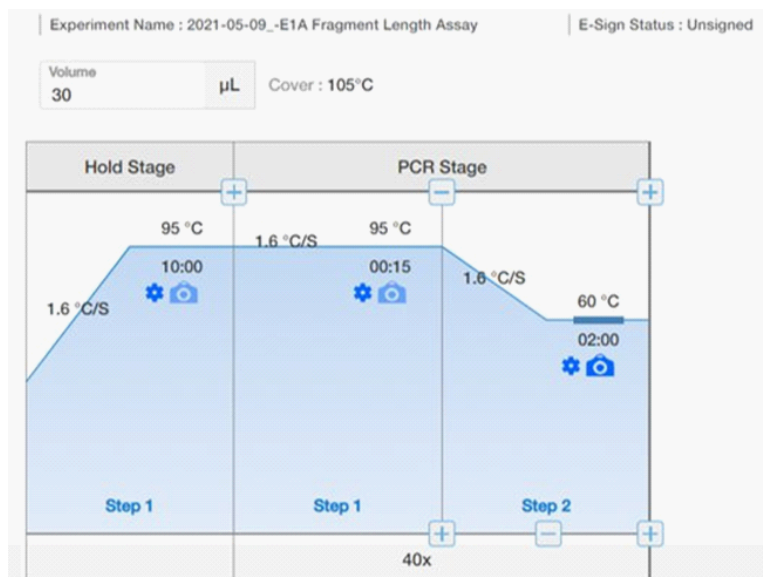
Template name cannot be changed after this step.

The qPCR Method screen is displayed.



## Edit the run method and optical filter selection

**Note:** This section provides general procedures to edit the run method and optical filter selection in the qPCR Method.



1. Ensure that the reaction volume is set to 30 µL.
2. Do not change **Step 1** of the **Hold Stage** in the default run method (thermal protocol).
  - To edit the default run method, see the *AccuSEQ™ Real-Time PCR Software v3.1 User Guide* (Pub. No. 100094287).

**Note:** Auto Delta and Veriflex functionality are not supported by the AccuSEQ™ Software v3.1.

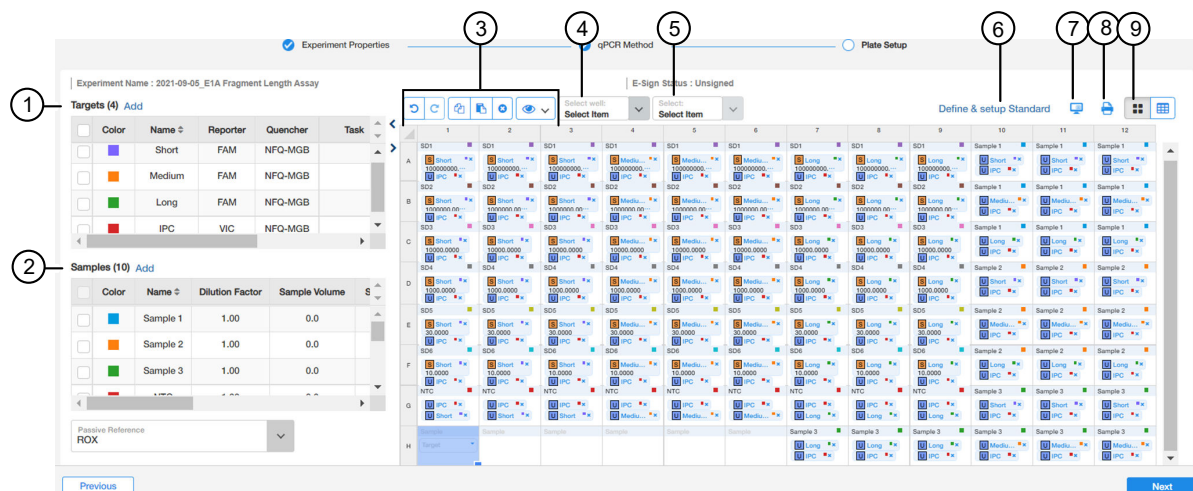
3. Change **Step 2** of the **PCR Stage** to 60°C for 2 minutes.
4. (Optional) Click **Optical Filter Settings** to view or edit the default filter settings.
  - The default optical filter selection is suitable for the resDNASEQ™ Quantitative E1A DNA Fragment Length Kit.
  - The resDNASEQ™ Quantitative E1A DNA Fragment Length Kit requires the QuantStudio™ 5 System to be calibrated for FAM™, VIC™ and ROX™ dyes.
  - For more information about system dyes and their calibration and optical filter selection, see *QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide* (Pub. No. MAN0010407).
5. Click **Next**.

## Assign plate and well attributes

**Note:** This section provides general procedures to set up the plate.

For specific instructions for each assay type, see the corresponding chapter in this guide. Do not change **Targets** for default assay templates.

1. In **Plate Setup** screen, click or click-drag to select plate wells in the **Grid View** of the plate.

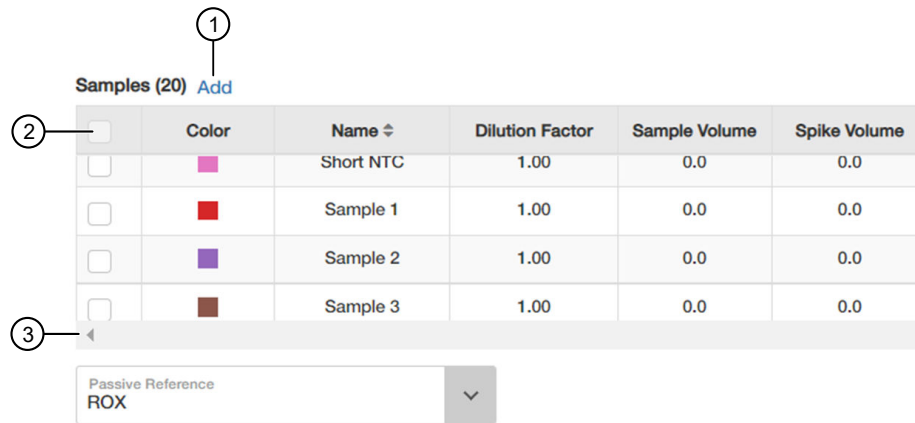


- 1 Targets
- 2 Samples
- 3 Plate setup toolbar
- 4 Select Item to highlight (Sample, Target, or Task).
- 5 Select Item. For example, Sample 1. Sample 1 replicates are highlighted.
- 6 Define & setup Standard
- 7 (View Legend)
- 8 (Print Preview)
- 9 (View (Grid View or Table View))

2. Assign the well attributes for the selected wells.

- To add new **Samples** or **Targets**, click **Add** in the appropriate column on the left of the screen, then edit the new Name and other properties as required. The new sample or target is then selectable within the wells of the plate.

**Note:** To change sample names, click the name in the Name column, then type the new name. To change Reporters and Quenchers, click the dye, then select from the dropdown list. Select the **FAM™** dye as the **Reporter** for E1A and the **VIC™** dye as the **Reporter** for IPC. Select the **NFQ-MGB** dye for the **Quencher**.



- ① **Add** button
- ② Checkbox—Select **Targets** and **Samples** to go in the selected well.
- ③ Scrollbar—Use to scroll to additional properties.

When a **Sample** or **Target** name are edited, two entries are added to the Audit trail (one for Delete, and another for Create).

- Use the plate setup toolbar (above the plate) to make edits to the plate.
    - Click **View** to show/hide the Sample Name, Sample Color, or Target from the view.
  - To add consecutive samples (with the same **Target**), select a well, then click-drag the dark blue box to the right.
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 3).
    - **Spike Standard Concentration**—expected spike amount per reaction (for example, 5,000 copies).
    - **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 copies per PCR reaction.


4. (Optional) Double-click a well to enter comments for the selected well.
5. Select the ROX™ dye from the **Passive Reference** dropdown list (bottom left of screen).
6. Click **Save** to save the template.  
This template can then be used to create experiments.

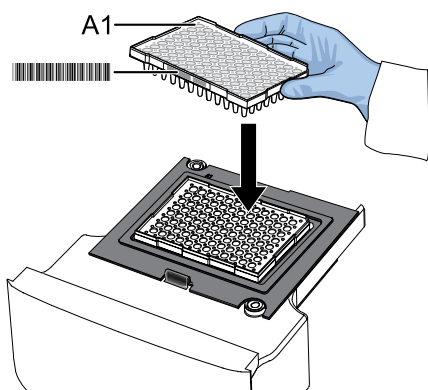
## Load the plate in the instrument



**CAUTION!** Use optical flat caps for tubes. Rounded caps can damage the heated cover.

Load the plate.

1. Touch  to eject the instrument drawer.
2. Load the plate onto the plate adapter so that:
  - Well A1 of the plate is in the top-left corner of the plate adapter.
  - The barcode faces the front of the instrument.



---

**IMPORTANT!** The instrument should be used by trained operators who have been warned of the moving parts hazard.


---

**Note:** Do not remove the black plate adapter before loading a plate or tube strips. If used, tube strips can fit loosely in the adapter, but the heated cover applies the appropriate pressure to seat the tube strips securely in the adapter.

---



**CAUTION! PHYSICAL INJURY HAZARD.** During instrument operation, the plate temperature can reach 100°C. Allow it to cool to room temperature before handling.

3. Touch  to close the instrument drawer.

## Start the run

Start the run in the AccuSEQ™ Software.

Option	Description
If the experiment is open	Click <b>Start Run</b> .
If the experiment is closed	<ol style="list-style-type: none"> <li>1. Open the experiment.</li> <li>2. Click the <b>Run</b> tab.</li> <li>3. Click <b>Start Run</b>.</li> </ol>

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


## Monitor the run

During the instrument run, you can monitor the run from the following places:

- On the instrument touchscreen.
- In the **Monitor the Run** pane of the AccuSEQ™ Software  (**Home**) screen.

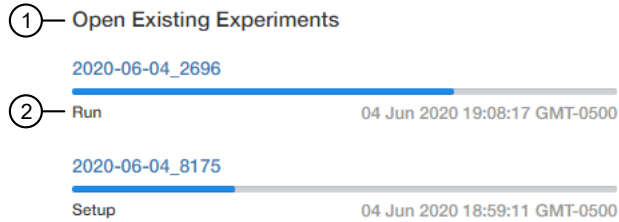


- ① Instrument name
- ② Instrument status (Ready, Running, Offline)
- ③ Calibration status
  - Calibrated—All required calibrations are "Current". Required calibrations include: Background, ROI/Uniformity, and Dyes.
  - Not calibrated—None of the required calibrations are complete.
  - Requires calibration—One or more dyes are not calibrated.
- ④ Time lapsed (if a run is in progress)
- ⑤ Total run time
- ⑥ Experiment name

- In the **Open Existing Experiments** pane of the AccuSEQ™ Software  (**Home**) screen. The experiment being run is the first experiment listed. Status is **Run**.

**Note:** You cannot start another run while the instrument status is **Running**.

When the run is complete, the status changes to **Analysis** and the status bar displays as complete.



① **Open Existing Experiments** pane

② Experiment status

- In the **Run** tab of the AccuSEQ™ Real-Time PCR Software.

You can perform the following actions.

- Select wells in the plate layout to highlight respective curves in the plot.
- Hover over curves in the plot for well information.
- (Optional) Change what is displayed in the table wells, by selecting **Sample Name**, **Sample Color**, or **Target** in the **View** dropdown list.

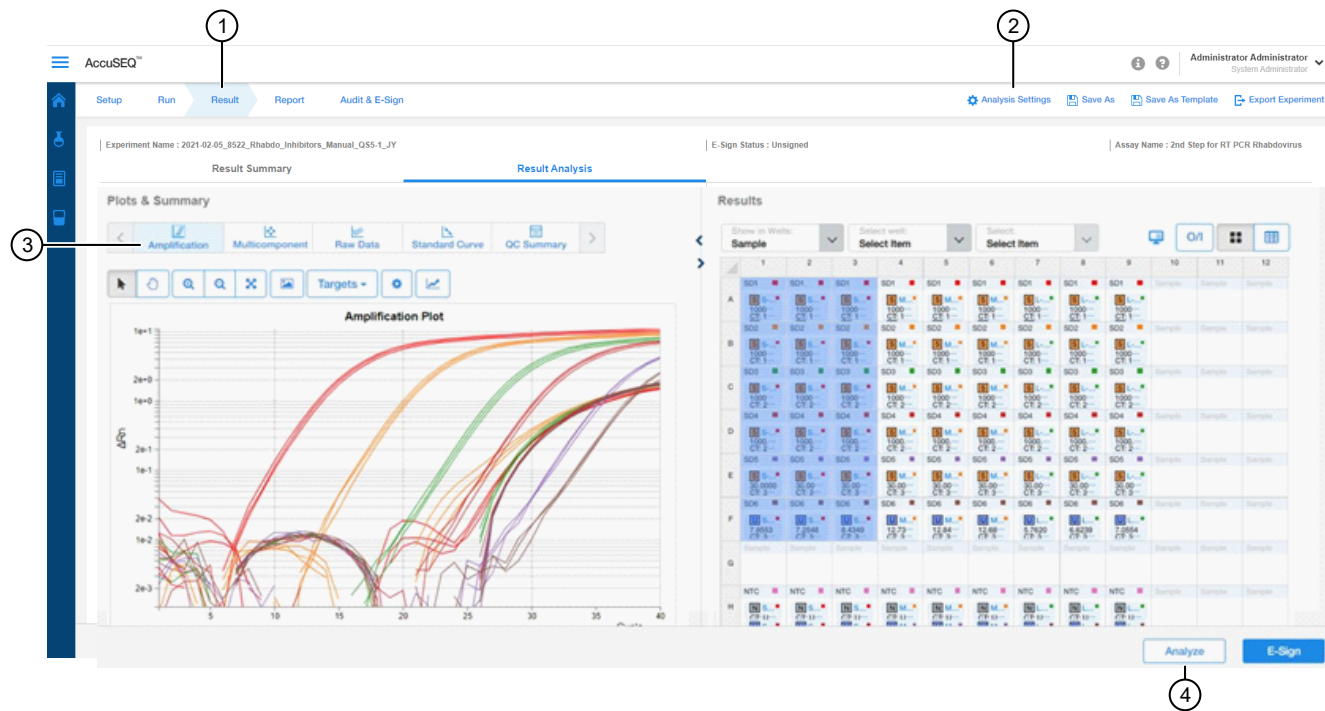
On run completion, the **Post Run Summary** displays the run length, user and instrument information, and a list of any errors that occurred.

When the run is complete, unload the plate from the instrument.

## Review the results

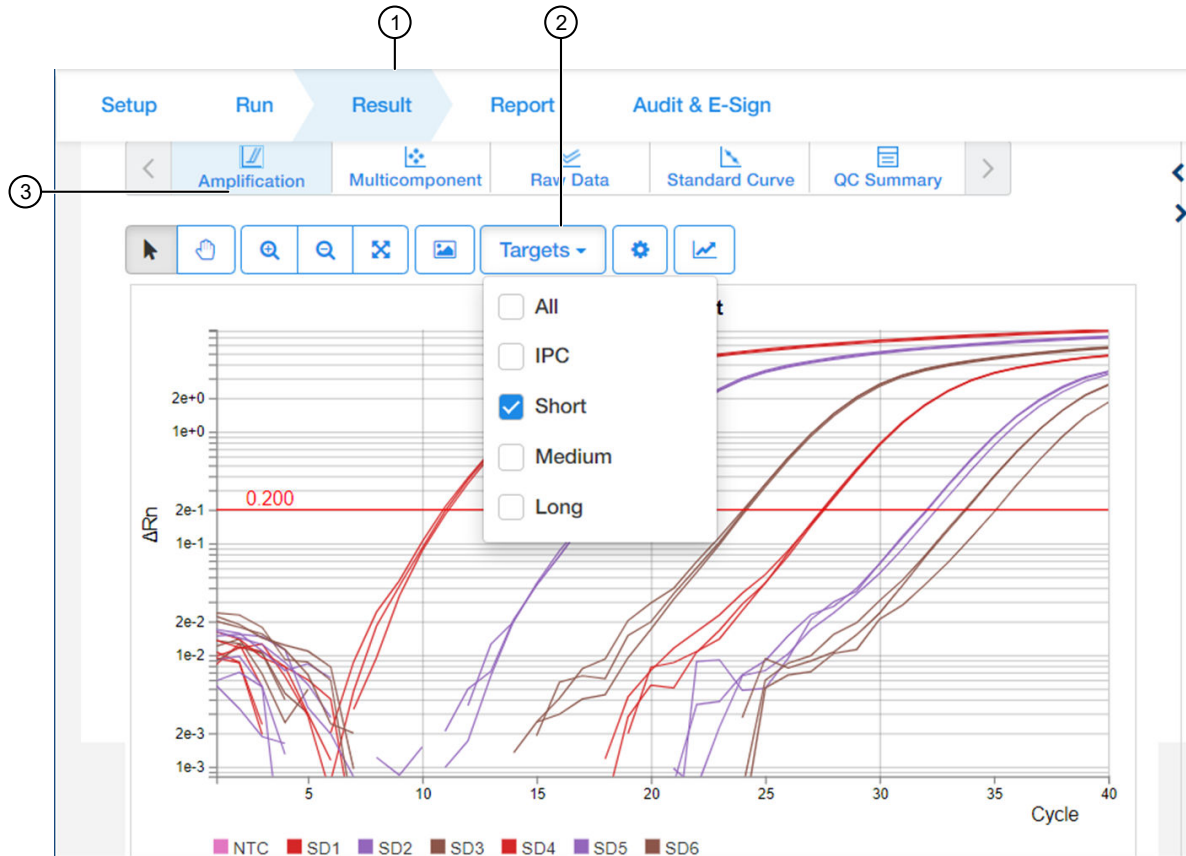
After the qPCR run is finished, use the following general procedure to analyze the results. For more detailed instructions see the *AccuSEQ™ Real-Time PCR Software v3.1 User Guide* (Pub. No. 100094287).

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

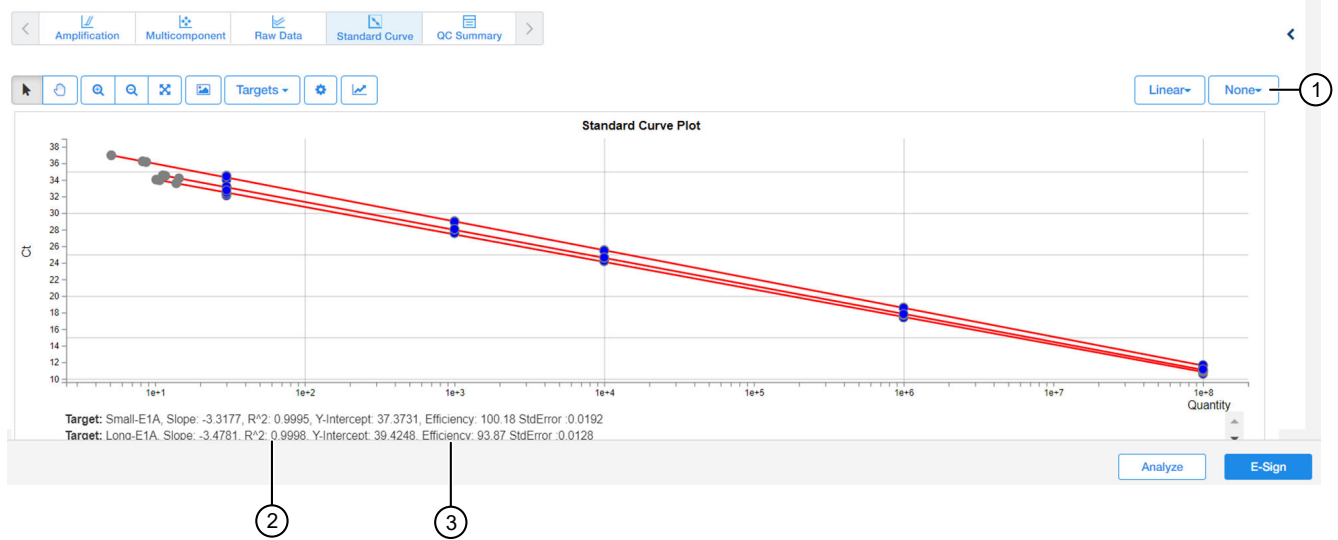
2. In the **Result Analysis** tab, review the Amplification Curve plots for amplification profiles in the controls, samples, and the standard curve. Ensure that threshold is set to 0.200.



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, R<sup>2</sup>, and Efficiency.



- ① Default plot settings
- ② R<sup>2</sup> value
- ③ PCR efficiency

**Note:** The **Standard Curve** efficiency should be between 90-110% and the R<sup>2</sup>>0.99. If these criteria are not met, up to two points, not in the same triplicate, can be removed from the standard curve data, and the analysis repeated.

5. (Optional) Select **File ▶ Print Report** to generate a hard copy of the experiment, or click **Print Preview** to view and save the report as a PDF or HTML file.
6. Export the results.
  - a. Navigate to the **Report** tab.
  - b. Check all boxes under **Contents**.
  - c. Select **Export Data in One File**.
  - d. Select the **XLS** format, then click **Export**.

**Note:** Users should determine criteria for their laboratory according to their laboratory protocols. Post data export, set all **UND** NTC values to 40 when calculating the average C<sub>t</sub>. For further guidance, see the United States Pharmacopeia (USP) General Chapter <509> Residual DNA Testing or regional regulatory guidance.

## Determine the copy number of E1A fragments

Download the **Fragment Length Calculation Tool** from the resDNASEQ™ Quantitative E1A DNA Fragment Length Kit product page to determine copy number and show the distribution percentage for each fragment length.

1. To determine the copy number with the **Fragment Length Calculation Tool**, navigate to the exported data for the experiment to be analyzed.
2. Copy, then paste the average copy number for each sample fragment length from the exported file to the **Raw Copy Number (From Std Curve out of qPCR software)** column of the tool.

Fragment Length Calculation Tool							ThermoFisher SCIENTIFIC	Read out
Sample	Target Length	Size range (bp)	Raw Copy Number (From Std Curve out of qPCR software)	Average Copy Number	Calculated Copy Number	Distribution (%)	Hard coded	
Replicate 1	Long	> 476	1025	1025.43	1025.43	32.07%	Data Input	
Replicate 2	Long		1095					
Replicate 3	Long		956					
Replicate 1	Medium	200 to 476	2701	2701.49	1676.06	52.42%		
Replicate 2	Medium		2835					
Replicate 3	Medium		2569					
Replicate 1	Short	<200	3197	3197.39	495.90	15.51%		
Replicate 2	Short		3046					
Replicate 3	Short		3349					

Figure 2 Fragment Length Calculation Tool with example data

To manually convert the copy number output to a mass measurement, multiply the copy number given by the AccuSEQ™ Real-Time PCR Software to the average molecular weight of a base pair (650g/mol), then divide by the Avogadro constant.

$$\text{Mass (ng)} = \frac{\text{Copy Number} \times [\text{Length of dsDNA} \times 1 \times 10^9 \times 650]}{6.022 \times 10^{23} \text{ mol}^{-1}}$$



# Troubleshooting

Observation	Possible cause	Recommended action
<p><math>\Delta Rn</math> and Ct values are inconsistent with replicates</p>	<p>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 the PrepSEQ™ workflow.</p>	<ol style="list-style-type: none"> <li>1. Select the <b>Component</b> tab. Confirm that affected wells generated significantly less fluorescence than unaffected replicates.</li> <li>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.</li> <li>3. For subsequent runs, ensure that the optical adhesive cover is correctly sealed to the reaction plate.</li> </ol>
	<p>Incorrect volume of PCR reaction mix was added to some reactions.</p>	<ol style="list-style-type: none"> <li>1. Select the <b>Component</b> tab. Confirm that affected wells generated significantly less fluorescence than unaffected replicates.</li> <li>2. Select the <b>Spectra</b> tab. Confirm that the wells with the incorrect volume of PCR reaction mix generated significantly different amounts of fluorescence than the unaffected wells.</li> <li>3. For subsequent runs, ensure the correct volume of PCR reaction mix.</li> </ol>
<p>Jagged amplification plots</p>	<p>Weak lamp or incorrect replacement.</p>	<p>Replace the lamp or make sure that the existing replacement is correct.</p>
<p>No defined amplification plots</p>	<p>An incorrect detector was selected on the amplification plot. <i>or</i> An incorrect detector was applied to the reactions when setting up the plate document.</p>	<ol style="list-style-type: none"> <li>1. Confirm that the correct detector was selected on the amplification plot.</li> <li>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.</li> </ol>
<p>Abnormal <math>\Delta Rn</math> values or negative <math>\Delta Rn</math> values</p>	<p>Incorrect passive reference was selected when setting up the plate document.</p>	<ol style="list-style-type: none"> <li>1. From the plate document, double-click a well to view the Well Inspector.</li> <li>2. Ensure that ROX™ dye was selected as the Passive Reference.</li> </ol>

Observation	Possible cause	Recommended 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. <i>or</i> The incorrect Quantity was entered. <i>or</i> Adjust baseline settings. <i>or</i> Poor standard curve preparation technique (forgot to mix, inaccurate pipetting).	<ol style="list-style-type: none"> <li>1. In the SDS software, from the plate document, double-click a well containing a DNA standard to view the Well Inspector.</li> <li>2. Ensure that the correct Task and Quantity are applied to the correct detector, then reanalyze.</li> <li>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 <math>R_n</math> vs <math>C_t</math> linear view.</li> <li>4. Increase final standard volumes (SD1 to SD6) up to 500 <math>\mu</math>L if required. Larger volumes will have less inaccuracies when pipetting.</li> </ol>
Standard curve for plasmid DNA 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 $C_t$ values of plasmid DNA samples	Incomplete vortexing of samples.	Repeat reactions, ensuring that samples and standards are vortexed for 15-30 seconds.
No target-specific signal (FAM dye) is detected in PCR positive control and/or extraction positive control wells	FAM™ dye detector not selected.	Make sure the FAM™ dye detector is selected in the analysis software. Repeat the analysis with the FAM™ dye detector selected.
No positive control signal (VIC dye) is detected in PCR positive control and extraction positive control wells	VIC™ dye detector not selected.	Make sure VIC™ dye detector is selected in the analysis software. Repeat the analysis with the VIC™ dye detector selected.
	Pipetting error (no positive control added).	Repeat the assay. Make sure to add positive control into all positive-control wells.
IPC signal is low	Inhibition of PCR or insufficient amount of assay mix added.	Repeat the sample preparation, then repeat the assay.  If PCR inhibition continues, contact your FAS, or dilute the sample (for example, 1:5 or 1:10) to dilute inhibitors.
No IPC signal is detected, but target-specific signal is detected	High copy number of target DNA resulting in preferential amplification of the target-specific DNA.	Report sample as positive.
No IPC signal is detected	The VIC™ dye detector was not selected.	Make sure the correct detector is selected in the analysis software. Repeat the analysis with the correct detector selected.

Observation	Possible cause	Recommended action
Target-specific signal or positive control signal is detected in negative control wells	Carryover contamination.	<ol style="list-style-type: none"> <li>1. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.</li> <li>2. If the negative control still continues to show contamination, contact Technical Support.</li> </ol>
Amplification plot crosses the threshold but no target-specific signal is amplified	Probe hydrolysis or non-optimal baseline setting.	<p>View amplification plots for the affected wells using <math>R_n</math> vs. <math>C_t</math> to confirm no up-tick in amplification.</p> <p>Change baseline settings. Check manual or autobaseline.</p> <p>Contact Technical Support.</p>
No dye signal in test sample well	Error in preparation.	Repeat the sample preparation and perform a new PCR reaction, using freshly prepared sample and reagents.
NTC signals are $< SD5$ (LOQ)	Contamination of master mix, assay mix, pipettes, or work area from improper handling of samples or control template.	Decontaminate the work area and use clean, dedicated pipettes when preparing reaction mixes. Where possible, carry out experiment in a biosafety cabinet or on dedicated work benches.



# 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](http://thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](http://fisherscientific.com) or another major laboratory supplier.

Item	Source
<b>Instrument</b>	
7500 Fast Real-Time PCR System with AccuSEQ™ software v2.x	Contact your local sales representative
<b>Generic consumables</b>	
Disposable gloves	MLS
Aerosol-resistant pipette tips	MLS
Pipettors: <ul style="list-style-type: none"><li>• Positive-displacement</li><li>• Air-displacement</li><li>• Multichannel</li></ul>	MLS
<b>Consumables for the 7500 Fast Real-Time PCR System</b>	
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL, 20 plates; for use with 7500 Fast Real-Time PCR System	<a href="#">4346906</a>
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	<a href="#">4314320</a>
MicroAmp™ Optical 8-Cap Strips, 300 strips	<a href="#">4323032</a>
MicroAmp™ Optical Adhesive Film Kit, 20 covers, 1 applicator, 1 optical cover compression pad	<a href="#">4313663</a>
MicroAmp™ Optical Adhesive Film	<a href="#">4360954</a>

## 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	E1A targets (short, medium, and long)	FAM™ dye (Select NFQ_MGB dye for quencher)
	IPC	VIC™ dye (Select NFQ_MGB dye 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: 2:00
Analysis	E1A targets (short, medium, and long)	Automatic Baseline Threshold: 0.2

## 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™** dye and quencher **NFQ\_MGB** dye.
  - 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™** dye and quencher **NFQ\_MGB** dye.
  - 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.

Tube label	Row-wells	Task	Quantity (copies/μL)
SD 1	A1–A9	Standard	1.00E + 08
SD 2	B1–B9	Standard	1.00E + 06
SD 3	C1–C9	Standard	10,000
SD 4	D1–D9	Standard	1,000
SD 5	E1–E9	Standard	30
SD 6	F1–F9	Standard	10

9. Assign the IPC to the standard curve wells.

	Short			Medium			Long					
	1	2	3	4	5	6	7	8	9	10	11	12
A	SD1	SD1	SD1	SD1	SD1	SD1	SD1	SD1	SD1	SA1	SA1	SA1
B	SD2	SD2	SD2	SD2	SD2	SD2	SD2	SD2	SD2	SA1	SA1	SA1
C	SD3	SD3	SD3	SD3	SD3	SD3	SD3	SD3	SD3	SA1	SA1	SA1
D	SD4	SD4	SD4	SD4	SD4	SD4	SD4	SD4	SD4	SA2	SA2	SA2
E	SD5	SD5	SD5	SD5	SD5	SD5	SD5	SD5	SD5	SA2	SA2	SA2
F	SD6	SD6	SD6	SD6	SD6	SD6	SD6	SD6	SD6	SA2	SA2	SA2
G	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	SA3	SA3	SA3
H	ERC	ERC	ERC	ERC	ERC	ERC	SA3	SA3	SA3	SA3	SA3	SA3

Figure 3 Example plate layout

- SD—Standard dilutions
- SA—Sample
- NTC—No template control
- ERC—Extraction control



10. In the **Run Method** screen, in the **Graphical View** tab.
  - a. In **Reaction Volume Per Well**, enter **30 µL**.
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**.
  - b. Select (highlight) both targets.
  - c. In the right-hand window, select **Use Default Settings**.
  - d. Click **Apply Analysis Settings**.
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, 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 3).
  - **Spike Standard Concentration**—expected spike amount per reaction (for example, 5,000 copies).
  - **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 copies 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**.

## Determine the copy number of E1A fragments

Download the **Fragment Length Calculation Tool** from the resDNASEQ™ Quantitative E1A DNA Fragment Length Kit product page to determine copy number and show the distribution percentage for each fragment length.

1. To determine the copy number with the **Fragment Length Calculation Tool**, navigate to the exported data for the experiment to be analyzed.
2. Copy, then paste the average copy number for each sample fragment length from the exported file to the **Raw Copy Number (From Std Curve out of qPCR software)** column of the tool.

Fragment Length Calculation Tool							ThermoFisher SCIENTIFIC	Read out
Sample	Target Length	Size range (bp)	Raw Copy Number (From Std Curve out of qPCR software)	Average Copy Number	Calculated Copy Number	Distribution (%)	Hard coded	
Replicate 1	Long	> 476	1025	1025.43	1025.43	32.07%	Data Input	
Replicate 2	Long		1095					
Replicate 3	Long		956					
Replicate 1	Medium	200 to 476	2701	2701.49	1676.06	52.42%		
Replicate 2	Medium		2835					
Replicate 3	Medium		2569					
Replicate 1	Short	<200	3197	3197.39	495.90	15.51%		
Replicate 2	Short		3046					
Replicate 3	Short		3349					

Figure 4 Fragment Length Calculation Tool with example data

To manually convert the copy number output to a mass measurement, multiply the copy number given by the AccuSEQ™ Real-Time PCR Software to the average molecular weight of a base pair (650g/mol), then divide by the Avogadro constant.

$$\text{Mass (ng)} = \frac{\text{Copy Number} \times [\text{Length of dsDNA} \times 1 \times 10^9 \times 650]}{6.022 \times 10^{23} \text{ mol}^{-1}}$$



# 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: 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	E1A targets (short, medium, and long)	FAM™ dye (Select <b>NFQ_MGB</b> dye for quencher)
	IPC	VIC™ dye (Select <b>NFQ_MGB</b> dye 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: 2:00
Analysis	E1A targets (short, medium, and long)	<b>Automatic Baseline or Manual Baseline</b> <sup>[1]</sup> Threshold: 0.2

<sup>[1]</sup> 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 reporter **FAM™** dye and quencher **NFQ\_MGB** dye.
  - c. Select a color for the detector, then click **Create Another**.
5. Click **New Detector**:
  - a. Enter **IPC** in the **Name** field.
  - b. Select reporter **VIC™** dye and quencher **NFQ\_MGB** dye.
  - c. Select a color for the detector, then click **OK**.
  - d. 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.

	Short			Medium			Long					
	1	2	3	4	5	6	7	8	9	10	11	12
A	SD1	SD1	SD1	SD1	SD1	SD1	SD1	SD1	SD1	SA1	SA1	SA1
B	SD2	SD2	SD2	SD2	SD2	SD2	SD2	SD2	SD2	SA1	SA1	SA1
C	SD3	SD3	SD3	SD3	SD3	SD3	SD3	SD3	SD3	SA1	SA1	SA1
D	SD4	SD4	SD4	SD4	SD4	SD4	SD4	SD4	SD4	SA2	SA2	SA2
E	SD5	SD5	SD5	SD5	SD5	SD5	SD5	SD5	SD5	SA2	SA2	SA2
F	SD6	SD6	SD6	SD6	SD6	SD6	SD6	SD6	SD6	SA2	SA2	SA2
G	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	SA3	SA3	SA3
H	ERC	ERC	ERC	ERC	ERC	ERC	SA3	SA3	SA3	SA3	SA3	SA3

Figure 5 Example plate layout

- SD—Standard dilutions
  - SA—Sample
  - NTC—No template control
  - ERC—Extraction control
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 (copies/μL)
SD 1	A1–A9	Standard	1.00E + 08
SD 2	B1–B9	Standard	1.00E + 06
SD 3	C1–C9	Standard	10,000
SD 4	D1–D9	Standard	1,000
SD 5	E1–E9	Standard	30
SD 6	F1–F9	Standard	10

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	Temperature	Time	Number of cycles
UNG incubation	95°C	10 minutes	1
Activation	95°C	15 seconds	40
Anneal/ extend	60°C	2 minutes	

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**.

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, 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**.



# 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.



## Good pipetting practices

- Use calibrated pipettes that are appropriate for volumes recommended by the manufacturer. Use pipette tips compatible with the pipettes.
- Immerse the pipette tip 2-3 mm below the meniscus during aspiration. Avoid air aspiration or pressing the tip on the container/tube bottom.
- Press and release the plunger at a consistent speed and pressure.
- Check the pipette tip before dispensing to ensure that there is appropriate volume drawn and that there is no additional droplet on the outside of the tip.
- When dispensing, press and hold the plunger to the second stop until tip is removed from the liquid. This prevents re-aspiration of the sample. Check the tip after dispensing to ensure that there is no residual liquid left in the tip.



**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.

## 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. 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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020  
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf>
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)  
[www.who.int/publications/i/item/9789240011311](http://www.who.int/publications/i/item/9789240011311)



# Documentation and support

## Related documentation

Document	Publication number	Description
<i>resDNASEQ™ Quantitative E1A DNA Fragment Length Kit Quick Reference</i>	MAN0025644	For brief instructions on using the resDNASEQ™ Quantitative E1A DNA Fragment Length Kit.
<i>resDNASEQ™ Quantitative DNA Kits User Guide</i>	4469836	For information on using resDNASEQ™ kits.
<i>PrepSEQ™ Sample Preparation Kits User Guide</i>	4469838	For information on preparing samples for extraction.
<i>PrepSEQ™ Residual DNA Sample Preparation Kit Quick Reference</i>	4469839	For brief instructions on preparing samples for extraction.
<i>AccuSEQ™ Real-Time PCR Software v3.1 User Guide</i>	100094287	For information on AccuSEQ™ Real-Time PCR Software v3.1 with the QuantStudio™ 5 Real-Time PCR System
<i>AccuSEQ™ Real-Time PCR Software v3.1 Quick Reference</i>	100094288	For basic information on AccuSEQ™ Real-Time PCR Software v3.1 with the QuantStudio™ 5 Real-Time PCR System
<i>AccuSEQ™ software: Custom Quick Reference Card</i>	4425585	For information on AccuSEQ™ software with the 7500 Fast instrument.
<i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve</i>	4347825	For information on the 7500 Fast instrument.

## Customer and technical support

Visit [thermofisher.com/support](https://thermofisher.com/support) for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments



- Order and web support
- 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](http://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](http://www.thermofisher.com/support).

