resDNASEQ™ Quantitative DNA Kits

For quantification of residual genomic and plasmid DNA from cell lines used in the bioproduction process

Catalog Numbers 4402085, 4458435, A46014, A26366, 4464335, 4458441, 4464336, A46066, A41797, A53242, and A50337

Pub. No. 4469837 Rev. K

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *resDNASEQ™ Quantitative DNA Kits User Guide* (Pub. No. 4469836). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare the control serial dilutions (Genomic DNA)

1. Label nonstick 1.5-mL microfuge tubes: **NTC, SD1**, **SD2**, **SD3**, **SD4**, **SD5**.

   For CHO, Vero, synthetic Vero, MDCK, and NS0 kits, label an additional tube with **SD6**.

2. Add 50 µL of DNA Dilution Buffer to tube NTC. Put aside.

3. Add 990 µL of DNA Dilution Buffer to tube SD1.

4. Add 450 µL of DNA Dilution Buffer to tubes SD2, SD3, SD4, SD5, and (for CHO, Vero, synthetic Vero, MDCK, and NS0 only) SD6.

5. Remove the tube of DNA control (30 ng/µL) from the freezer.

6. After the DNA thaws, vortex it gently for 2 seconds, then briefly centrifuge.

7. Perform the serial dilutions:

   Note: The Vero PLASMID DNA Control, supplied in the resDNASEQ™ Quantitative Synthetic Vero DNA Kit, should be vortexed for 15–30 seconds after each dilution.

   a. Add 10 µL of the DNA control to the tube that is labeled SD1, then vortex thoroughly and briefly centrifuge.

   b. Transfer 50 µL of the DNA from tube SD1 to tube SD2, then vortex thoroughly and briefly centrifuge.

c. Continue to transfer 50 µL of DNA from the previous dilution tube to the next dilution tube until you add DNA to tube SD5 (*E. coli*, HEK293, Human, *Pichia*, and Sf9 and Baculovirus) or SD6 (CHO, Vero, synthetic Vero, MDCK, and NS0). Final dilutions are shown in the table. After each transfer, vortex thoroughly, then centrifuge briefly.

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

8. Store the DNA dilution tubes:

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

Prepare the control serial dilutions (Plasmid DNA - Kanamycin Resistance)

1. Label nonstick 1.5-mL microfuge tubes: **NTC, SD1**, **SD2**, **SD3**, **SD4**, **SD5**, and **SD6**.

   The dilution **SD6** will not be used for the standard curve. It will be used to confirm the limit of detection (LOD).

2. Add 70 µL of DNA Dilution Buffer to tube NTC. Put aside.

3. Add 990 µL of DNA Dilution Buffer to tube Dilution 1.

4. Add 180 µL of DNA Dilution Buffer to tubes SD1, SD2, SD3, SD4, and SD5.

5. Add 100 µL of DNA Dilution Buffer to tube SD6.

6. Remove the tube of KanR DNA control (3.0 x 10^7 copies/µL) from the freezer.

7. After the DNA thaws, vortex it thoroughly for 15-30 seconds, then tap gently to bring contents to the bottom of the tube. Do not centrifuge.

8. Perform the serial dilutions:
   a. Add 10 µL of the KanR DNA control (3.0 x 10^7 copies/µL) to the tube that is labeled Dilution 1, vortex thoroughly for 15-30 seconds, then tap gently to bring contents to the bottom of the tube.
   b. Transfer 20 µL of the DNA from tube Dilution 1 to tube SD1, vortex thoroughly for 15-30 seconds, then tap gently to bring contents to the bottom of the tube..
   c. Continue to transfer 20 µL of DNA from the previous dilution tube to the next dilution tube until you add DNA to tube SD5.

d. Transfer 100 µL of DNA from SD5 to SD6, then vortex thoroughly. Final dilutions are shown in the table. After each transfer, vortex thoroughly for 15-30 seconds, then tap gently to bring contents to the bottom of the tube. Do not centrifuge.

Serial dilution (SD) tube	Dilution	Concentration (copy number/ µL)	Copy number/ PCR reaction
Control	DNA control tube	3.0×10^7	N/A
Dilution 1	10 µL DNA control + 990 µL DDB	300,000	N/A
SD 1	20 µL Dilution 1 + 180 µL DDB	30,000	300,000
SD 2	20 µL SD1 + 180 µL DDB	3,000	30,000
SD 3	20 µL SD2 + 180 µL DDB	300	3,000
SD 4	20 µL SD3 + 180 µL DDB	30	300
SD 5	20 µL SD4 + 180 µL DDB	3	30
SD 6 (LOD)	100 µL SD5 + 100 µL DDB	1.5	15

9. Store the DNA dilution tubes:

Temprature	For use
4°C	≤2 days
–20°C	Dilution 1 ≤1 week
–20°C	Dilution 1 in single-use aliquots ≤6 months

Prepare the PCR reaction mix

1. Determine the number of reactions needed for the controls and test samples that you will quantify.
2. Thaw all kit reagents completely at room temperature, thoroughly mix reagents, and briefly centrifuge.
3. Prepare a PCR reaction mix using the reagents and volumes shown in the table below.
   - Multiply the PCR reaction volume for one reaction (30 µL) by the number of reactions that you need to run.
   - Use 10% excess volume to compensate for pipetting losses.

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

Kit reagents	Volume for 1 30-µL reaction	Volume for 36 30-µL reactions (includes 10% overage)
Negative Control (Water)	2 µL	79.2 µL
10X DNA assay mix appropriate for the cell line being tested	3 µL	118.8 µL
TaqMan™ Environmental Master Mix 2.0	15 µL	594 µL
DNA template	10 µL	Add DNA template to each well separately, not as part of Master Mix
Total	**30 µL**	**792 µL** of Master Mix

Prepare the PCR plate

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

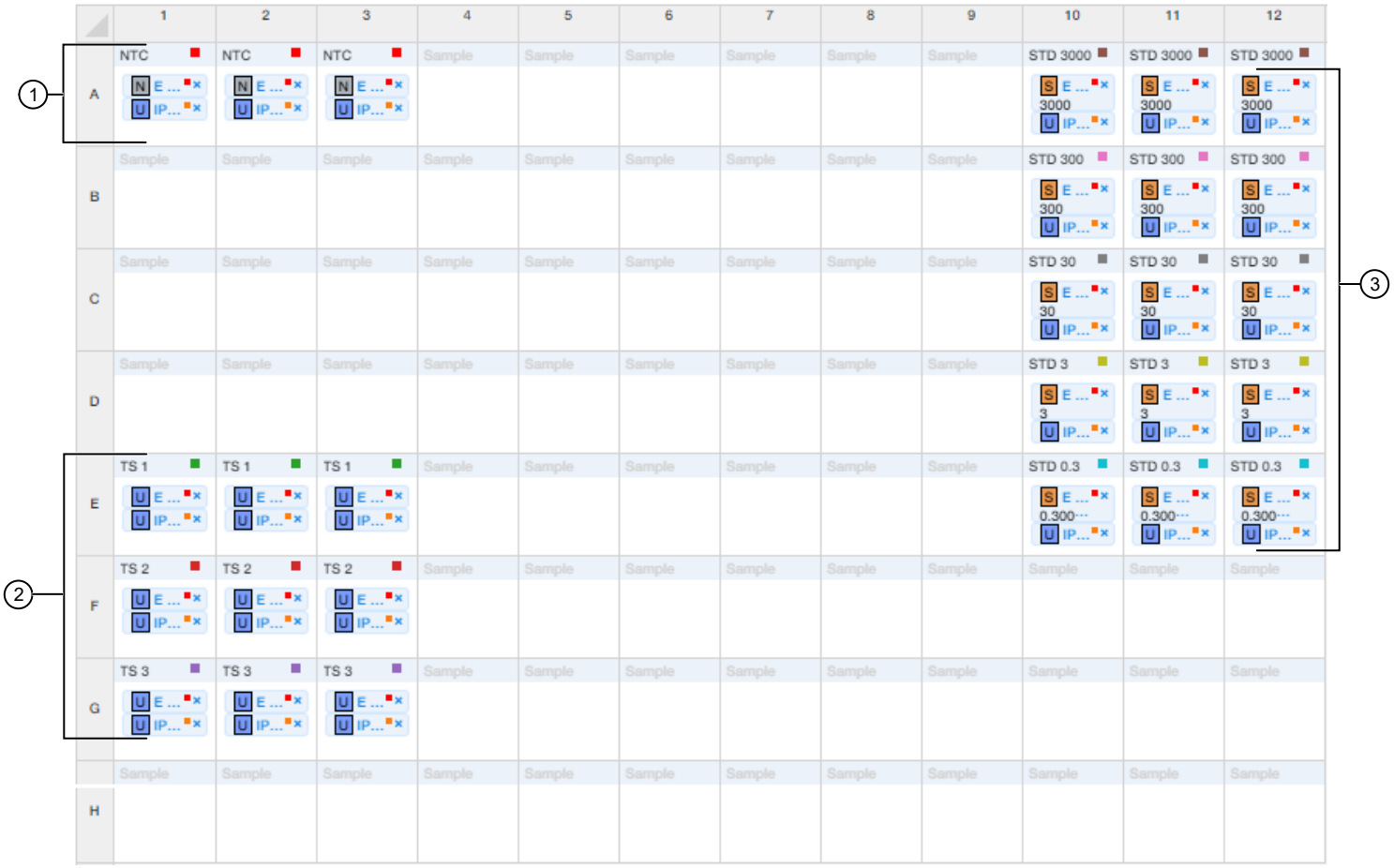


Figure 1 Default plate setup in the AccuSEQ™ Real-Time PCR Software v3.1

① No template controls
② Samples
③ Standard curve

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

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

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

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

Create a resDNASEQ™ experiment (Genomic DNA)

1. In the **Home** screen, click the **Factory default/Admin Defined Template** tab, then select a **resDNASEQ** template.
2. In the **Experiment Properties** pane of the **Setup** tab:
   a. (*Optional*) Change the system-generated name of the experiment.

      Note: Names must be unique. Deleted experiment names can not be reused.

   b. (*Optional*) Enter the plate **Barcode**, then add **Comments**.

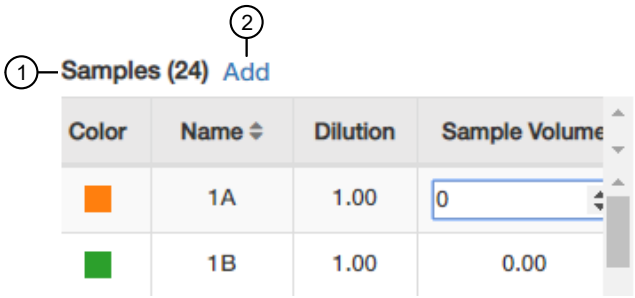
      Note: Comments are not editable post analysis.

      Default resDNASEQ™ settings (cannot be changed)
      - **Experiment Type** is **Quantitation-Standard Curve**
      - **Chemistry** is **TaqMan™ Reagents**
      - **Ramp Speed** is **Standard - 2hrs**

   c. Click **Next**.

      Note: Experiment names cannot be changed after this step.
3. In the **qPCR Method** pane of the **Setup** tab, view the default volume and cycling conditions (cannot be changed).
4. Click **Next**.
5. In the **Samples** pane of the **Setup** tab, enter the sample **Name** and **Dilution**. **Sample Volume** is not applicable. Add additional **Samples** if needed.

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



① **Samples** pane

② **Add**—adds additional samples

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

   For more information on plate setup, see *AccuSEQ™ Real-Time PCR Software v3.1 User Guide* (Pub. No. 100094287).
7. Click **Next**.

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

   Note: Clicking **Save As** will create a copy of the experiment.
9. Assemble the PCR reactions following the manufacturer's instructions for the reagents and following the plate layout set up in the template. See *resDNASEQ™ Quantitative DNA Kits User Guide* (Pub. No. 4469836).

Create a resDNASEQ™ template (Plasmid DNA)

Plasmid DNA resDNASEQ™ assays do not use a **Factory default/Admin Defined Template**. Create a new template before the first use of these assays.

1. In the **Home** screen, click **Templates** in the left navigation pane.
2. Click **Create New** next to the **ResDNA_5Std** factory default template.
3. Click **Next** to move to the **qPCR Method** screen.
4. Click **Next** to move to the **Plate Setup** screen.
5. In the **Plate Setup** screen, add the **Targets** and **Reporters**.
6. Click **Save as Template**.
7. Enter a **Template Name** and description, then select **Admin Defined** and **Locked**. Click **Save**.
   The template is saved, and can be accessed from **Templates** in the **Home** screen.
8. Click **Templates** in the **Home** screen, then open the new template.
   Note: The template must be saved prior to editing the **Analysis Settings**
9. Click **Analysis Settings**, then deselect **Default Settings**.
10. Enter new thresholds for the targets.

    Table 1 resDNASEQ™ Quantitative Plasmid DNA - Kanamycin Resistance Gene Kit

Target	Threshold
Kanamycin	0.04
IPC	0.02

11. Click Apply, then close the template.
12. In the **Home** screen, click **Templates** in the left navigation pane, then **Publish** the template. See *AccuSEQ™ Real-Time PCR Software v3.1 User Guide* (Pub. No. 100094287).
    The template is listed in the **Factory default/Admin Defined Templates**.

Create a resDNASEQ™ experiment (Plasmid DNA)

Plasmid DNA resDNASEQ™ assays do not use a **Factory default/Admin Defined Template**. Create a new template before the first use of these assays.

1. In the **Home** screen, click **Factory default/Admin Defined Templates**, then select the custom plasmid DNA **resDNASEQ** template created in “Create a resDNASEQ™ template (Plasmid DNA)” on page 6.

   Note: To create an experiment from an existing resDNASEQ™ experiment, see *AccuSEQ™ Real-Time PCR Software v3.1 User Guide* (Pub. No. 100094287).

2. In the **Experiment Properties** pane of the **Setup** tab:
   a. (*Optional*) Change the system-generated name of the experiment.

      Note: Names must be unique. Deleted experiment names can not be reused.

   b. (*Optional*) Enter the plate **Barcode**, then add **Comments**.

      Note: Comments are not editable post analysis.

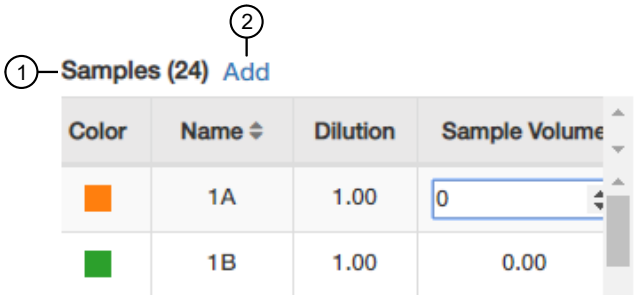
      Default resDNASEQ™ settings (cannot be changed)
      - **Experiment Type** is **Quantitation-Standard Curve**
      - **Chemistry** is **TaqMan™ Reagents**
      - **Ramp Speed** is **Standard - 2hrs**

   c. Click **Next**.

      Note: Experiment names cannot be changed after this step.

3. In the **qPCR Method** pane of the **Setup** tab, view the default volume and cycling conditions (cannot be changed).
4. Click **Next**.
5. In the **Samples** pane of the **Setup** tab, enter the sample **Name** and **Dilution**. **Sample Volume** is not applicable. Add additional **Samples** if needed.

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



① **Samples** pane

② **Add**—adds additional samples

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

   For more information on plate setup, see *AccuSEQ™ Real-Time PCR Software v3.1 User Guide* (Pub. No. 100094287).
7. Click **Next**.

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

   Note: Clicking **Save As** will create a copy of the experiment.
9. Assemble the PCR reactions following the manufacturer's instructions for the reagents and following the plate layout set up in the template. See *resDNASEQ™ Quantitative DNA Kits User Guide* (Pub. No. 4469836).

Start the run

Start the run in the AccuSEQ™ Software.

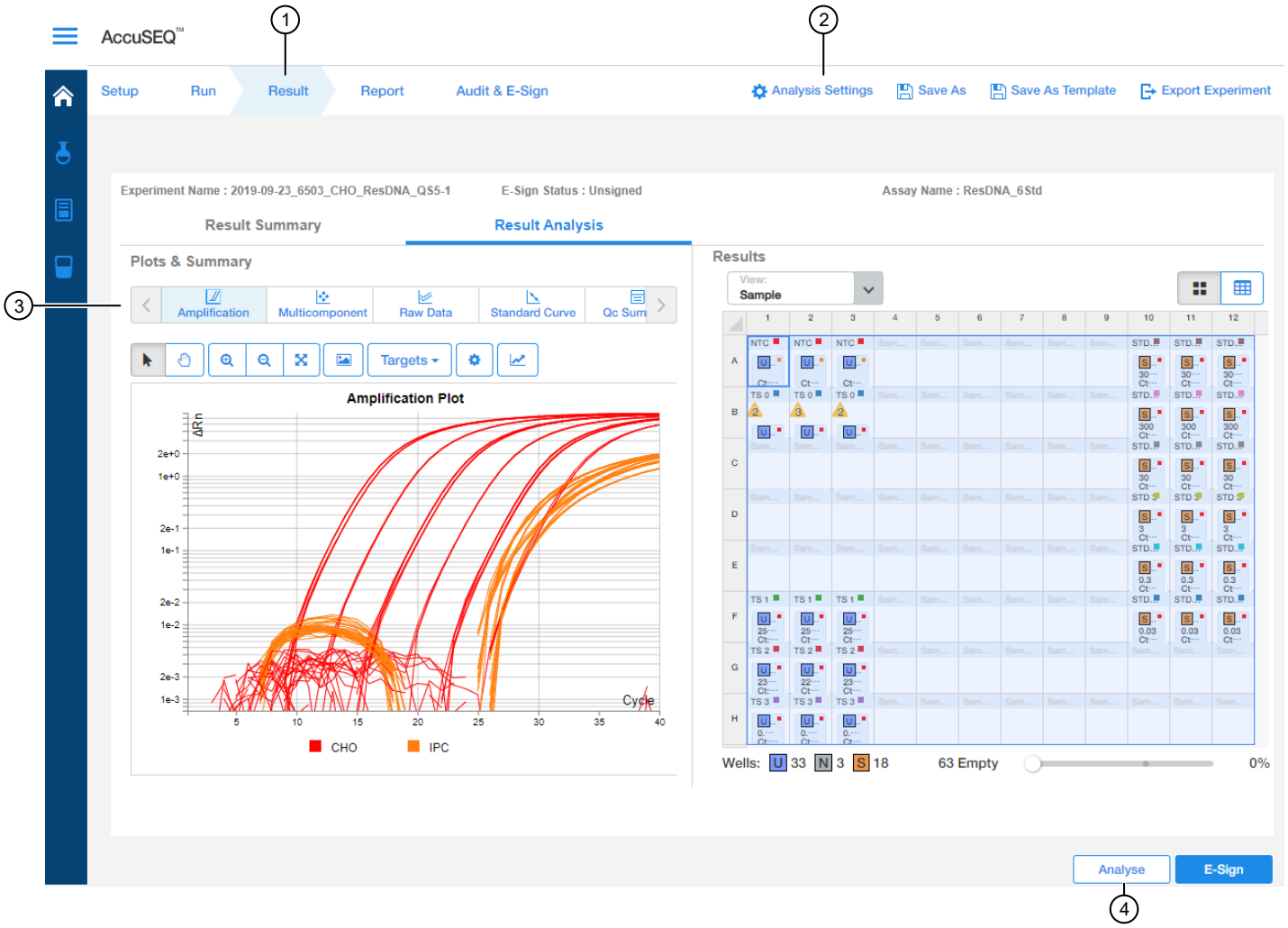
Option	Description
If the experiment is open	Click **Start Run**.
If the experiment is closed	1. Open the experiment. 2. Click the **Run** tab. 3. Click **Start Run**.

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

Analyze the results

After the qPCR run is finished, use the following general procedure to analyze the results. For more detailed instructions see the *AccuSEQ™ Real-Time PCR Software v3.1 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 auto threshold is selected.

   Note: (Plasmid- KanR assay only) Ensure that the threshold was set to 0.04 for Kanamycin target and 0.02 for IPC.

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^2, and Efficiency.

   Note: The **Standard Curve** efficiency should be between 90-110% and the R^2>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*) Navigate to the **Report** tab to generate a report of the experiment, or to export results.

6. (*Optional*) 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 the plasmid, then divide by the Avogadro constant.

$$Mass\ (g) = \frac{Copy\ number\ x\ Molecular\ weight\ (gmol^{-1})}{6.0221\ X\ 10^{23}\ mol^{-1}}$$

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Revision history: Pub. No. 4469837

Revision	Date	Description
K	8 November 2021	Update to include the resDNASEQ™ Quantitative Synthetic Vero DNA Kit (Cat. No. A53242).
J	28 April 2021	Update to the control serial dilutions required for the resDNASEQ™ Quantitative Plasmid DNA - Kanamycin Resistance Gene Kit (Cat. No. A50337).

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