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Use the AccuSEQ® Software v2.0 Custom Experiment mode

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Custom Experiments

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *AccuSEQ® Software v2.0 Mycoplasma Experiments Getting Started Guide (PN 4425587)*. For every chemical, read the safety data sheet and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Overview

About AccuSEQ® Software v2.0

AccuSEQ® Software v2.0 is data acquisition and analysis software used with Real-Time PCR (polymerase chain reaction) assays.

This quick reference card provides abbreviated procedures for creating, analyzing, viewing, and interpreting quantitation experiments using the Custom Experiment mode of AccuSEQ® Software v2.0. The quantitation of DNA, where samples were prepared using the resDNASEQ CHO DNA kit, will be used as an example of abosolute quantitation.

About Custom Experiments

Use the AccuSEQ® Software v2.0 Custom Experiment mode to design, run, and analyze your own standard curve, melt curve, or presence/absence experiment. Steps in the workflow include selecting the experiment type, designating tasks and targets, setting thermal cycling conditions, and performing data analysis. Both data collection and data processing are fully editable in the Custom Experiment mode workflow.

For automated data processing of presence absence experiments for Mycoplasma, use the Mycoplasma SEQ module.

Related documents

- *AccuSEQ® Software Help* – Access the Help system by pressing **F1**, by clicking the help icon in the toolbar of the AccuSEQ® Software window, or by selecting **Help ▸ Contents and Index**.
- *resDNASEQ® Quantitative CHO DNA Kit Protocol* (Pub. no. 4415260)

Example files

Example files shipped with the software:

- Standard curve files shipped with the software:
  - **Example experiment** — CHO residual DNA Quantitation Example.eds
  - **Experiment template** — CHO residual DNA Quantitation Example.edt

Custom Experiment workflow

The following flowchart summarizes the steps for performing a typical Custom Experiment workflow using the AccuSEQ® Software v2.0.

Step 1: Set up the experiment (page 2)
Create an experiment.
Define the experiment properties.
Set up the plate.
Define the run method.
Review the reaction setup.

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Step 2: Prepare the reactions (page 4)

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Step 3: Run the experiment (page 4)
Prepare for the run.
Start the run.
(Optional) Monitor the run.

▼

Step 4: Review the results (page 4)
Analyze the experiment.
Review the QC Summary.

▼

Step 5: Review the plots (page 5)
Review the standard curve.
Review the amplification plot.

▼

Step 6: (Optional) Modify the experiment (page 6)
Edit analysis settings.

▼

Step 7: (Optional) Export and print the data (page 7)
Export data.
Print a report.

Step 1: Set up the experiment

Create an experiment

1. In the desktop, double–click (**AccuSEQ®** Software) to start the software.
2. Log in to the software. See your system administrator for username and password.

   Note: If you have Administrator privileges, you can enable or disable the Security, Audit, and E-Sig (SAE) settings for experiments. For more information, see the *AccuSEQ® Software v2.0 Help*.
3. In the Home screen ( ), click **Create Custom Experiment** to open the Custom Experiment workflow.

   For the example DNA absolute quantitation experiment, you can also choose to create a new custom experiment using the setup information from the CHO experiment template file (*CHO residual DNA Quantitation Example.edt*) that is shipped with AccuSEQ® Software v2.0. For more information, see the *AccuSEQ® Software v2.0 Help*.

Define the experiment properties

In the Experiment Properties screen:

1. Click the **Experiment Name** field and enter up to 100 characters (excluding / \ > < * ? " | : ;) to identify the experiment.
2. *(Optional)* Click the **Barcode** field and enter up to 100 characters to identify the barcode on the reaction plate.
3. *(Optional)* Click the **Comments** field and enter up to 1000 characters to describe the experiment.
4. Select the remaining experiment properties: experiment type, reagents, and ramp speed.

   For the example DNA absolute quantitation experiment:

Experiment property	Selection
Experiment type	Quantitation - Standard Curve
Reagents	TaqMan® Reagents
Ramp speed	Standard (~2 hours to complete a run)

Set up the plate

Before you run the experiment, verify that the instrument has been correctly calibrated during installation and properly maintained on the recommended calibration schedule. Refer to the *Applied Biosystems® 7500/7500 Fast Real-Time PCR Systems Maintenance Guide* (Pub. no. 4387777) or *AccuSEQ® Software v2.0 Help*.

Define targets

Define the targets to detect and quantify in the reaction plate.

1. In the navigation pane, select **Setup ▸** **Plate Setup**.
2. On the Plate Setup screen, select the **Define Targets and Samples** tab.
3. Click **Add New Target** in the toolbar above the target table.
4. Define the targets.

   For the example standard curve experiment:

Target Name	Reporter	Quencher	Color†
CHO	FAM™	NFQ-MGB	
IPC	VIC®	NFQ-MGB	

† The target color is associated with the target in the plate layout, analysis plots, reports, and exported data.

Define samples

Define the samples to test in the reaction plate.

1. In the **Define Targets and Samples** tab, click **Add New Sample** in the toolbar above the sample table.
2. Define the samples.

   The example standard curve experiment requires 12 total samples:

Sample Name	Color†
Enter the following series: Sample 1, Sample 2, Sample 3, Sample 4, Sample 5, SC1, SC2, SC3, SC4, SC5, and SC6	Use the defaults
NTC	

† The sample color is associated with the sample in the plate layout, analysis plots, reports, and exported data.

Define and set up standards

Enter the number of points and replicates for all standard curves in the reaction plate. For each standard curve, enter the starting quantity and select the serial factor.

1. On the Plate Setup screen, select the **Assign Targets and Samples** tab.
2. Click **Define and Set up Standards** below the target table.
3. Complete the Define and Set up Standards dialog box.

   For the example standard curve experiment:

Field/Selection	Entry
Target	CHO
# of Points	6
# of Replicates	3
Starting Quantity	0.03 (pgDNA/reaction)
Serial Factor	10x

Field/Selection	Entry
Use Wells	1. Select option: Let Me Select Wells 2. Select wells in plate layout: A10-A12, B10-B12, C10-C12, D10-D12, E10-E12, F10-F12
Arrange standards in	Rows

4. Click **Apply**, verify the standard curve set up in the plate layout, then click **Close**.

Assign targets and samples

Assign targets and samples to wells in the plate layout, and select the passive reference.

1. In the **Assign Targets and Samples** tab, select wells using the plate layout or the table view.
2. Assign samples to wells: Select the **Assign** checkbox (indicated by a ☑) next to the sample you want to assign to the selected wells.

   Note: You can assign only one sample to a well.
3. Assign targets to wells:

   a. Select the **Assign** checkbox (indicated by a ☑) next to the target you want to detect and quantify in the selected wells.

   Note: You cannot assign more than two targets to a well.

   b. Select the detection task for the selected wells.

   For the example standard curve experiment, make the following assignments:

Well(s)	Sample	Target	Target Task
A4-A6	Sample 1	• CHO • IPC	U †
B4-B6	Sample 2		
C4-C6	Sample 3		
D4-D6	Sample 4		
E4-E6	Sample 5		
A10-A12	SC 6	CHO	S ‡
B10-B12	SC 5		
C10-C12	SC 4		
D10-D12	SC 3		
E10-E12	SC 2		
F10-F12	SC 1		
H7-H9	NTC	CHO	N §
		IPC	U †

† **Unknown** (default) - The selected wells contain samples with unknown quantities of target.
‡ **Standard** - The selected wells contain samples with known standard quantities (see "Define and set up standards" for settings).
§ **Negative Control** - The selected wells contain water or buffer instead of sample and should contain no target.

4. Select a dye to use as the passive reference.

   For the example standard curve experiment, use the default selection (**ROX**™ dye).

View the plate layout

The Plate Layout tab displays information about each well in the reaction plate in an illustration.

As needed, in the Plate Layout screen:

- Change the display of the plate layout.
- Move a sample to a new well position.
- Save the plate layout as an image.
- Print the plate layout.

Define the run method

1. In the navigation pane, select **Setup** ▸ **Run Method**.
2. Select the **Graphical View** tab, review the reaction volume and thermal profile, then edit as needed.

   For the example standard curve experiment:

   - Set the Reaction Volume Per Well to **30 µL**.
   - Edit the default run method: On the Run Method screen, click the first Holding Stage in the thermal profile, then click **Delete Selected** in the run method toolbar to remove the stage from the thermal profile.

Review the reaction setup

1. In the navigation pane, select **Setup** ▸ **Reaction Setup**.
2. For each target to detect in the reaction plate, select the assay type (if using TaqMan® reagents), then review the components and calculated volumes for preparing the standard dilution series, samples, and real-time PCR reactions. If needed, edit the reaction volume, excess reaction volume, component concentrations, and/or stock concentrations.
3. Click **Print Reaction Setup** to print instructions on how to prepare the real-time PCR reactions.

   Set the printed reaction setup instructions aside until you prepare the reactions (see "Step 2: Prepare the reactions").

Note: For resDNASEQ assay experiments, including the example standard curve experiment, use the *resDNASEQ® Quantitative CHO DNA Kit Protocol* (Pub. no. 4415260) for reaction setup instructions.

Save the experiment setup

1. To save the experiment, select a Save option from the toolbar:
   - **Save**
   - **Save As**
   - **Save As Template**

2. Enter the file name, then select a location for the experiment.

3. Click **Save**.

   Specify a reason for change, if prompted. For more information, see the *AccuSEQ® Software Help*.

IMPORTANT! Do not open, edit, or manipulate the experiment files stored on the hard drive of the computer outside of AccuSEQ® Software v2.0. If you do so, you will corrupt the experiment file and you will not be able to open it in AccuSEQ® Software v2.0.

Step 2: Prepare the reactions

For more details, refer to the manufacturer's instructions for the reagents you are using.

For the example DNA absolute quantitation experiment, prepare the following reactions according to the *resDNASEQ® Quantitative CHO DNA Kit Protocol* (Pub. no. 4415260):

- Template
- Sample dilutions
- Standard dilution series
- Reaction mix
- PCR reactions

Step 3: Run the experiment

Prepare for the run

Open the experiment

1. Select **File ▸ Open** from the toolbar to open the experiment you saved when you set up the experiment.

2. In the dialog box that opens, select the experiment, then click **Open**.

(Optional) Enable the notification settings

If you want the system to send email notifications about the instrument run:

1. In the navigation pane, select **Run ▸ Notification Settings**.

2. Select **Yes** to enable notifications, then define the notification settings. See the *AccuSEQ® Software v2.0 Help* for more information.

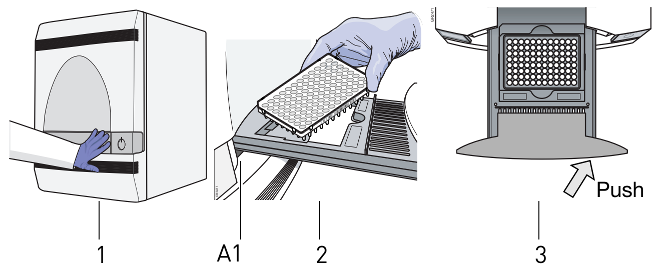
Start the run

Load the instrument

1. Push the tray door to open it.

2. Load the plate into the plate holder in the instrument with the notched A1 position at the top-left of the tray. Ensure that the plate is properly aligned in the holder.

3. Push the tray door to close it. Apply pressure to the right side of the tray door at an angle.



Start the run

1. In the navigation pane, select **Run**.

2. Click **Start Run** (green button at the top of every run screen).

   Specify a reason for change, if prompted.

(Optional) Monitor the run

1. In the navigation pane, select **Run**.

2. Select a run screen from the navigation pane to monitor the progress:

   - **Amplification Plot** - Select to view the change in normalized reporter signal (ΔRn) for each cycle.
   - **Temperature Plot** - Select to view the change in sample and block temperature (°C) throughout the run.
   - **Run Method** - Select to view the run method defined on page 3.

   At the top of every run screen, you can view the run status.

Unload the instrument

When your 7500 Fast system displays the Run Complete message, unload the reaction plate from the instrument.

1. Push the tray door to open it.

2. Remove the reaction plate.

3. Push the tray door to close it.

Step 4: Review the results

Analyze the experiment

1. If not already open, open an experiment that contains run data.

2. Select **Analysis** from the navigation pane.

3. If the data are not analyzed, click **Analyze**.

4. In the navigation pane, select an analysis screen to view the data (for example, select **QC Summary** to view a quality summary of the data).

Review the QC Summary

The QC Summary displays a list of the AccuSEQ® Software v2.0 flags, the flag frequency, and location of the wells associated with the flag. Use the QC Summary to review the flags applied to wells of the reaction plate and to view troubleshooting information.

1. In the navigation pane, select **Analysis** ▸ **QC Summary**.
2. Review the Flag Summary.

   In the example standard curve experiment, there are no flagged wells .
3. In the Flag Details table, look in the Frequency and Wells columns to determine which flag(s) appear in the experiment.

   For custom standard curve experiments, the flags listed below may be triggered by the experiment data.

Flag	Description
AMPNC	Amplification in negative control
BADROX	Bad passive reference signal
BLFAIL	Baseline algorithm failed
CTFAIL	C_T algorithm failed
EXPFAIL	Exponential algorithm failed
HIGHSD	High standard deviation in replicate group
OFFSCALE	Fluorescence is offscale
NOAMP	No amplification
NOISE	Noise higher than others in plate
NOSIGNAL	No signal in well
OUTLIERRG	Outlier in replicate group
SPIKE	Noise spikes
THOLDFAIL	Thresholding algorithm failed
MTP	Multiple T_m peaks (if the experiment includes a melt curve)

   In the example standard curve experiment, the Frequency column displays 0.
4. *(Optional)* Click each flagged row to display details about the flag and highlight the flagged well(s) in the plate layout or well table.
5. To further investigate results, select any of the following Analysis screens:
   - Table View tab in each analysis option (this page)
   - Standard Curve (see page 6)
   - Amplification Plot (see page 6)
   - Melt Curve (if the experiment includes a melt curve)
   - Presence/Absence Plot (presence/absence experiments only)
   - *(Optional)* Multicomponent Plot
   - *(Optional)* Raw Data Plot

Review the table data

The Table View tab displays data for each well in the reaction plate in a table format.

For custom absolute quantitation experiments, this data includes:

- The well position, sample name, target name, task, and dyes
- The calculated threshold cycle (C_T), C_T mean, and C_T SD
- CV %
- % difference
- Back calculated value
- Quantity values, quantity mean, and quantity SD
- Comments
- Flags

For information on the data displayed in the Table View for other experiment types, see the *AccuSEQ® Software v2.0 Help*.

To review the table data:

1. In the navigation pane, select **Analysis**, then select the **Table View** tab.
2. Select well(s) in the Table View:
   - To select a well, click a row in the well table.
   - To select multiple wells:
     - **Click-drag** or **Shift-Click** to select continuous rows in the well table. **Ctrl-Click** to select discontinuous rows in the well table.
     - Use the **Group By** drop-down list to group wells by a specific category.
     - Use the **Select Wells With** drop-down lists to filter wells by a specific category.

   Selected wells display in the corresponding plot(s).

For the example standard curve experiment, review the table data for:

- Unknown sample quantity values
- C_T values (including C_T standard deviation)
- Flags

Step 5: Review the plots

Review the following plots for custom absolute quantitation experiments. For information on the available plots for other experiment types, see the *AccuSEQ® Software v2.0 Help*.

After reviewing plots, you can:

- Edit analysis settings (see page 6).
- Omit wells from analysis (see page 6).
- Export data (see page 7).
- Print a report (see page 7).
- Print SAE reports (see page 7).
- Sign the results.

Review the standard curve

The Standard Curve screen displays the standard curve (as C_T values vs. standard quantities) for samples designated as standards in the reaction plate. AccuSEQ® Software v2.0 calculates the quantity of an unknown target from the standard curve.

The Table View of the Standard Curve screen reports Back Calculated Value, % difference, C_T Mean, and C_T SD.

Plot settings offer target, plot color, fit (including non-linear regression types), and weighting (only for non-linear regression).

To review the Standard Curve:

1. In the navigation pane, select **Analysis** ▸ **Standard Curve**.
2. Display all 96 wells in the Standard Curve screen by clicking the upper left corner of the plate layout in the Plate Layout tab.
3. Verify the regression coefficient values that are displayed below the standard curve. The displayed values are based on the type of regression selected. Eff% and Slope are displayed for Linear regression. A, B, C, and D are displayed for 4PL. A, B, C, D, and E are displayed for 5PL.
4. Verify that all unknown samples (blue dots) are between the lowest concentration and highest concentration on the standard curve (red dots).
5. Select the **Table View** tab, then use the Group By drop-down list to review the C_T values.

Review the amplification plot

The amplification plot displays the data collected during the cycling stage of PCR amplification. It can be viewed as:

- Baseline-corrected normalized reporter (ΔRn) vs. cycle
- Normalized reporter (Rn) vs. cycle
- Threshold cycle (C_T) vs. well

To review the Amplification Plot:

1. From the navigation pane, select **Analysis** ▸ **Amplification Plot**.
2. Display all 96 wells in the amplification plot by clicking the upper left corner of the plate layout in the Plate Layout tab.
3. Use the Plot Settings tab to review the amplification plot for:
   - C_T values
   - Irregular amplification
   - Outliers

Step 6: *(Optional)* Modify the experiment

Edit analysis settings

Note: You must have administrator or scientist privileges to edit analysis settings.

If the default analysis settings in the AccuSEQ® Software v2.0 are not suitable for your Custom Experiment, you can change the settings in the Analysis Settings dialog box after a run completes, then reanalyze your experiment.

1. Open an analyzed Custom Experiment.
2. Select **Analysis** ▸ **Analysis Settings** from the toolbar. The Analysis Settings dialog box displays the analysis settings used for the current experiment.
3. Edit the default analysis settings as needed.

   In the example DNA absolute quantitation experiment, the default analysis settings are used in the:
   - **C_T Settings tab** – Threshold (0.2) and baseline (3-15 cycles) settings by target
   - **Flag Settings tab** – Flag sensitivity and use
   - **Advanced Settings tab** – Baseline settings by well

   For information on the analysis settings used for the example standard curve experiment, see the *resDNASEQ® Quantitative CHO DNA Kit Protocol* (Pub. no. 4415260). For information on the available analysis settings for other experiment types, see the *AccuSEQ®* Software *v2.0 Help*.
4. Click **Apply Analysis Settings**.
5. Click **Analyze** to reanalyze the data with the new analysis settings.

   Specify a reason for change and sign the experiment, if prompted.

Omit wells from the analysis

You may omit wells from analysis if you do not want to consider data generated by the well.

1. Open an analyzed Custom Experiment.
2. From the navigation pane, select **Analysis**.
3. Using the plate layout or table view in any Analysis screen, select one or more wells to omit from analysis (for example, an outlier in a replicate group).
4. Right-click the well(s), then select **Omit**. Results for the selected well(s) are removed.
5. Click **Analyze** to reanalyze the data without the omitted well(s).

   Specify a reason for change and sign the experiment, if prompted.

Step 7: *(Optional)* Export and print the data

Export data

1. In the toolbar of an analyzed Custom Experiment, click **Export**.
2. Complete the tasks on the Export Properties tab:
   a. Select the data to export:
      - Sample Setup
      - Raw Data
      - Amplification Data
      - Results
      - Multicomponent Data
   b. Select to export all data in one file or in separate files for each data type.
   c. Enter export file properties, then click **Start Export**.
      Specify a reason for change and sign the experiment, if prompted.
3. Click ☒ to close the Export Data dialog box.

Print a report

1. In the toolbar of an analyzed Custom Experiment, click **Print Report**.
2. Select the data to include in the report. The data available vary according to the Custom Experiment type.
   For custom standard curve experiments, the report can include:
   - Experiment Summary
   - Results Summary
   - Plate Layout
   - Amplification Plot (ΔRn vs. Cycle, Rn vs. Cycle, C_T vs. Well)
   - Standard Curves
   - Results Table (By Well)
   - QC Summary
3. Select a print option:
   - **Print Preview** – Preview, save, or print the report.
   - **Print** – Send the instructions to a printer.
     Specify a reason for change and sign the experiment, if prompted.
4. Click ☒ to close the Print Report dialog box.

Print SAE reports

You can print SAE reports for the current experiment in the Experiment screen: Audit Report or E-Sig Report.

For more information, see the *AccuSEQ® Software v2.0 Help*.

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