Use the AccuSEQ® Software v2.0 Mycoplasma SEQ module

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Mycoplasma SEQ Experiments

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

Overview

About the AccuSEQ® Software v2.0

Life Technologies™ AccuSEQ® Software v2.0 is detection and analysis software used with Real-Time PCR (polymerase chain reaction) assays for impurity and contaminant analysis in pharmaceutical quality control and process development environments. This quick reference card provides abbreviated procedures for creating, analyzing, viewing, and interpreting Mycoplasma SEQ experiments using AccuSEQ® Software v2.0.

Related documents

- *AccuSEQ® Software v2.0 Help* – Access the Help system by pressing **F1**, by clicking in the toolbar of the AccuSEQ® Software v2.0 window, or by selecting **Help ▸ AccuSEQ Software Help**.
- Documents shipped with the software:
  - *AccuSEQ® Software Mycoplasma Experiments Getting Started Guide* (Pub. no. 4425587)
  - *Use the AccuSEQ® Software v2.0 Custom Experiment mode Quick Reference Card* (Pub. no. 4425585)
  - *MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit* product insert (Pub. no. 4482248)

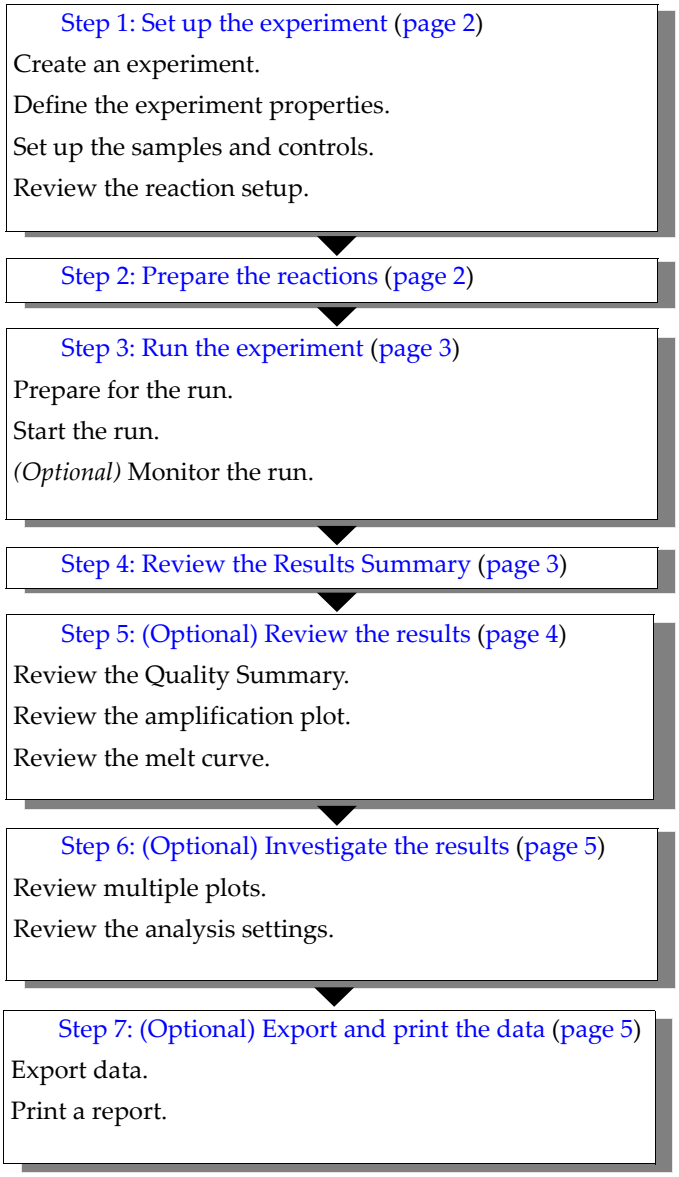
About Mycoplasma SEQ experiments

The SEQ Experiment workflow calculates reagent volumes, determines optimum plate setup, guides you through experiment set up, uses optimized thermal cycling conditions, and performs data analysis. The Mycoplasma SEQ experiment workflow is designed to set up, run, and analyze samples prepared with the MycoSEQ® *Mycoplasma* Real-Time PCR Detection Kit. Perform a Mycoplasma SEQ experiment to detect the presence of mycoplasma species nucleic acid sequence in a sample.

IMPORTANT! The Mycoplasma SEQ experiment performs a presence/absence assay and analyzes melt curve data. The SEQ presence/absence assay and the melt curve algorithm are customized for detection of specific organisms and species and may yield different results than a custom presence/absence experiment or a custom melt curve experiment.

Mycoplasma SEQ experiment workflow

The following flowchart summarizes the steps for performing a typical Mycoplasma SEQ experiment workflow using AccuSEQ® Software v2.0.



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. You can also edit analysis settings. For more information, see the *AccuSEQ®* Software v2.0 *Help*.
3. In the Home screen, click **Create SEQ Experiment** to open the SEQ Experiment workflow.
4. In the SEQ assay selection dialog, verify that **Mycoplasma Assay v2.0** is selected from the presence/absence assay group, then click **Next**.

Define the experiment properties

Experiment, reagents, run method, and target(s) are defined by the software based on the SEQ assay selected when you created the experiment.

In the Experiment Properties screen:

1. Enter an experiment name that is descriptive and easy to remember.
2. *(Optional)* Enter a barcode to identify the barcode on the reaction plate.
3. *(Optional)* Enter comments to describe the experiment.
4. Verify the SEQ experiment type and assay to use, then click **Next**.

Set up the samples and controls

In the Sample Setup screen:

1. Specify the number of samples and replicates:

Field	Minimum Entry†
Samples	1
Sample replicates	1
Inhibition control replicates for each sample	0
Positive control replicates	1
Negative control replicates	1

† Use at least one negative and one positive control per run, and at least one inhibition control per sample.

2. Set the Sample volume per reaction (2-10 µL).

   Note: Life Technologies recommends a sample volume per reaction of 10 µL for Mycoplasma SEQ experiments. The 7500 Fast system supports total reaction volumes from 10 to 30 µL.
3. Enter sample names.
4. *(Optional)* Set plot colors.
5. Click **Next**.

Review the reaction setup

The AccuSEQ® Software v2.0 uses the sample information that you enter in the Sample Setup screen to fill the wells in the plate layout (see below) and to calculate the required reaction component volumes for each sample type, based on the *Mycoplasma Real-Time PCR Detection Kit* guidelines.

View the plate layout

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

Review and adjust the plate layout (click-drag or Swap wells) as needed, then click **Next**.

IMPORTANT! If you want to modify the default plate layout, refer to the *Mycoplasma* Real-Time PCR Detection Kit for plate layout suggestions.

Print the experiment setup

Print the experiment setup for use in reaction preparation.

1. In the Print Experiment Setup screen, select the elements to include in the report:
   - Detailed Instructions
   - Plate Layout
2. Click **Preview**, **Save As PDF** (or HTML), or **Print**.

Finish the experiment setup

Click **Save & Finish** in the open experiment screen, specify a name and location for the .eds file, then click **OK**.

Specify a reason for change, if prompted. For more information, see the *AccuSEQ®* Software v2.0 *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 a Mycoplasma SEQ experiment, you prepare PCR reactions that contain primers designed to amplify the mycoplasma targets and a reagent to detect amplification of the targets.

1. Prepare the PCR reactions as described in the printed reaction setup worksheet.
2. Pipette samples into the plate according to the printed plate layout.

   For details on plate sealing, see the *Mycoplasma* Real-Time PCR Detection Kit.

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

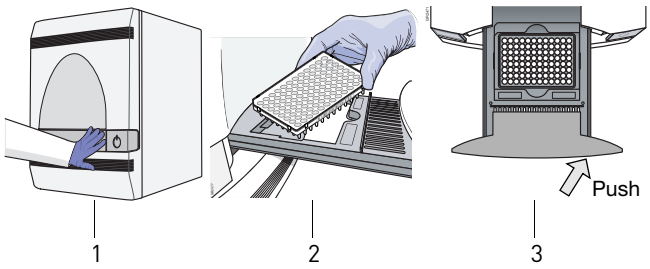
If you want the system to send email notifications about the instrument run:

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** - View the change in normalized reporter signal (ΔRn) for each cycle.
   - **Melt Curve** - View the first derivative of the normalized reporter signal (-Rn) plotted against temperature (°C).
   - **Temperature Plot** - View the change in sample and block temperature (°C) throughout the run.
   - **Run Method** - View the default run method.

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

   Note: During a Mycoplasma SEQ experiment run, progress bars reflect run status. During the third stage of a Mycoplasma SEQ experiment, there is a delay between melting and completion of the stage while the system analyzes the data.

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 Summary

Use the Call Summary, Plate Layout and Table views in the Results Summary screen to review the experiment results.

1. From the navigation pane, select **Results** ▸ **Results Summary**.
2. Review the Call Summary for results:

Sample Type	Call	Description
Positive Control Inhibition Control Negative Control	Pass	Target passed.
	Fail	Target failed.
Unknown	Present	Target signal is present and is within the temperature range, derivative range, and C_T range for the assay.
	Fail	Target failed.
	Absent	Target signal is not present within the temperature range, derivative range, and C_T range for the assay.
	Review	The target failed. Review the result.

As needed:

- To review any fail or review results, review the Quality Summary (see page 4).
- Export and print the data (see page 5).
- Sign the results.

Step 5: *(Optional)* Review the results

Review the Quality Summary

Use the Quality Summary to review the call assessments applied to wells of the reaction plate and view troubleshooting information. This summary includes the call assessment frequency and location for the open experiment.

1. Click **Results** in the navigation pane, then click **Quality Summary**.
2. In the Quality Summary table, look in the Frequency and Wells columns to determine which call assessments appear in the experiment.

   For Mycoplasma SEQ experiments, the call assessments listed below may be triggered by the experiment data.

   - All positive controls failed
   - All negative controls failed
   - Bad passive reference signal
   - Baseline algorithm failed
   - C_T algorithm failed
   - Exponential algorithm failed
   - Failed IC prevents call
   - High background signal
   - Inhibition detected
   - NC criteria failed
   - No signal in well
   - Not supported by IC
   - PC criteria failed
   - Positive control signal detected
   - Review C_T and DV values
   - Thresholding algorithm failed
3. *(Optional)* Click a call assessment in the table to display more information about the assessment and highlight the associated well(s) in the plate layout or table view.

As needed:

- To further investigate any fail or review results, review the following:
  - Amplification Plot (see below)
  - Melt Curve (see page 4)
  - Table data (see page 4)
- Export and print the data (see page 5).
- Sign the results.

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. Click **Plot Analysis** in the navigation pane, then click **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

Review the melt curve

The Melt Curve plot displays the fluorescence peak data collected at each well position during the melt curve stage of an instrument run. For Mycoplasma SEQ experiments, AccuSEQ® Software v2.0 determines the melting temperature (T_m) and derivative value (DV) for each peak.

To review the Melt Curve:

1. Click **Plot Analysis in the navigation pane, then click Melt Curve**.

   IMPORTANT! The T_m values displayed below the melt curve correspond to the largest peak in the target range. To determine the target T_m, review the table data (see below). The T_m values displayed below the plot may not exactly match the values displayed in the table view because of rounding and/or interpolation.
2. Select wells in the plate layout to view in the melt curve plot.

Review the table data

The Table View displays results data for each well in the reaction plate, including:

- The well number, sample name, sample type, call, call assessment, and cycle 1 fluorescence (FSU)
- The calculated values: C_T, target T_m , and ΔRn

  Note: The cycle 1 fluorescence and ΔRn values are only available for display in the Table View when it is accessed from the Plot Analysis screens.

To review the table data:

1. In the navigation pane, select **Results** or **Plot Analysis**, then select the **Table View** tab.
2. Select well(s) in the Table View to display in the corresponding plot(s).
3. Review the following values for wells with review or fail results:
   - C_T
   - ΔRn
   - Target T_m

Step 6: *(Optional)* Investigate the results

Review multiple plots

1. Click **Plot Analysis** in the navigation pane, then click **Multiple Plots View**.
2. Select **Multicomponent Plot** and **Raw Data Plot**.
3. Click a sample in the plate layout or the table view to display the corresponding plots.

Review the analysis settings

The Analysis Settings dialog box displays the pre-defined threshold cycle (C_T) analysis settings for Mycoplasma SEQ experiments, optimized in the AccuSEQ® Software v2.0 for use with the MycoSEQ® *Mycoplasma* Real-Time PCR Detection Kit.

If the pre-defined analysis settings are not suitable for your experiment, you can change the settings in the Analysis Settings dialog box, then reanalyze your experiment.

1. Select **Analysis** ▸ **Analysis Settings** from the toolbar menu.
2. Define the C_T settings, call settings, and QC and noise settings to use for the Mycoplasma target in the table. See the *AccuSEQ® Software v2.0 Help* for more information.
3. Click **Apply Analysis Settings**.
4. Click **Analyze** to reanalyze the data with the new C_T settings.

   Specify a reason for the 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. In the navigation pane, select **Plot Analysis**.
2. Using the plate layout or table view, select one or more wells to omit from analysis.
3. Right-click the well(s), then select **Omit**. Results for the selected well(s) are removed.
4. 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 Mycoplasma SEQ experiment, click **Export**.
2. Complete the tasks on the Export Properties tab:
   a. Select the Mycoplasma SEQ data to export:
      - Sample Setup
      - Results
      - Raw Data
      - Amplification Data
      - 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 Mycoplasma SEQ experiment, click **Print Report**.
2. Select the Mycoplasma SEQ data to include in the report:
   - Experiment Summary
   - Results Summary
   - Plate Layout
   - Amplification Plot (ΔRn vs. Cycle, Rn vs. Cycle, C_T vs. Well)
   - Melt Curve (Derivative Reporter, Normalized Reporter)
   - Quality Summary
3. Click **Preview**, **Save As PDF** (or HTML), or **Print**.

   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*.

Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**

lifetechnologies.com

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