

## Streck ARM-D<sup>®</sup> Kits

# Data Acquisition and Analysis Guide

**Real-Time PCR Platform:  
Applied Biosystems QuantStudio 7 Flex Real-Time PCR System**

### Quick Links

[General Recommendations](#)

[Instrument Set-up](#)

[Data Analysis and Interpretation: Streck ARM-D Kit, \*ampC\*](#)

[Data Analysis and Interpretation: Streck ARM-D Kit,  \$\beta\$ -Lactamase](#)

[Data Interpretation: Unknown Samples](#)

[Troubleshooting](#)

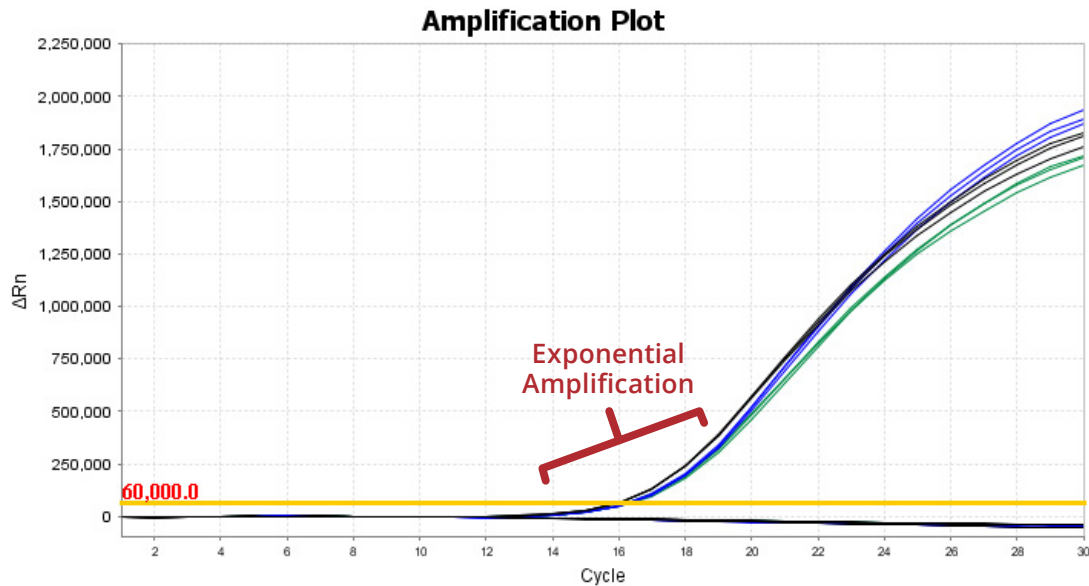
This guide is intended to be used as a Streck ARM-D Kit-specific supplement for the Instructions For Use (IFU) document included with each kit. The Streck ARM-D kits referenced in this document are labeled as CE IVD and are For Export Only. Not for sale in the U.S. The instructions provided in this guide serve as set-up and analysis guidelines which were determined during the validation of the Streck ARM-D Kits. Certain settings may be changed as needed to optimize data analysis following a PCR run. Refer to the instrument manual for a detailed description on the instrument's operation and data analysis.

### General Recommendations

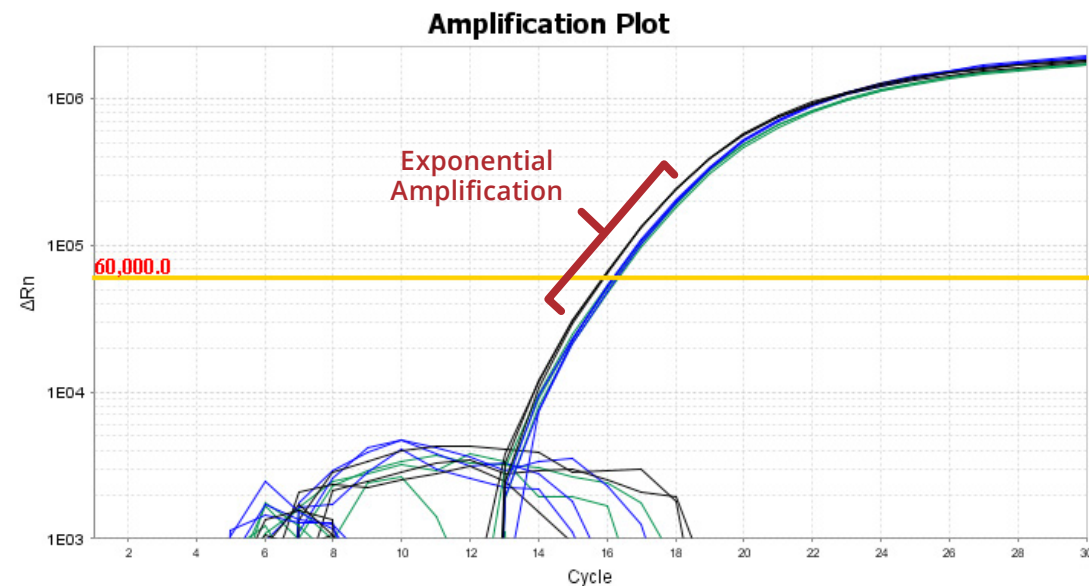
**Instrument and Protocol Set-up:** A template protocol can be made and reused for future assays to reduce instrument set-up time after the first run with each kit.

**Threshold Settings:** Although automatic analysis is often appropriate, manually setting threshold values is more convenient for consistent comparisons between runs. Recommended settings for fluorophore-specific thresholds (Tables 1 and 3) are provided in this document, based on data acquired during product validation. However, these values may be adjusted after reviewing data or changed to improve analysis of a specific target. To maximize the precision and sensitivity of the assay, threshold values should be set in the linear phase of exponential amplification and above baseline RFU levels. This can be done by viewing the log plot and moving the threshold line for each target and/or fluorophore within the linear phase of the log plot and above background (see the following examples).

## Linear Scale View



## Log Scale View



**Baseline Settings:** Similar to the threshold settings, automatic baseline settings often give acceptable results, but manually defining the baseline Start and End cycles may help avoid software errors that could affect data interpretation. These guidelines provide recommended values for the baseline cycle settings based on typical Cq values obtained during kit validation when using the same DNA concentrations as described in the IFU (10-200 ng/ $\mu$ L of bacterial DNA) and may be necessary to adjust following data evaluation. To adjust the baseline cycles manually for each fluorophore or target, decide which reaction is the first with fluorescence that exceeds the visible baseline level; then determine the cycle at which the fluorescence signal starts to increase in the sample. Adjust the baseline end cycle to 3 cycles prior to the earliest amplification and repeat the same steps for the rest of the targets.

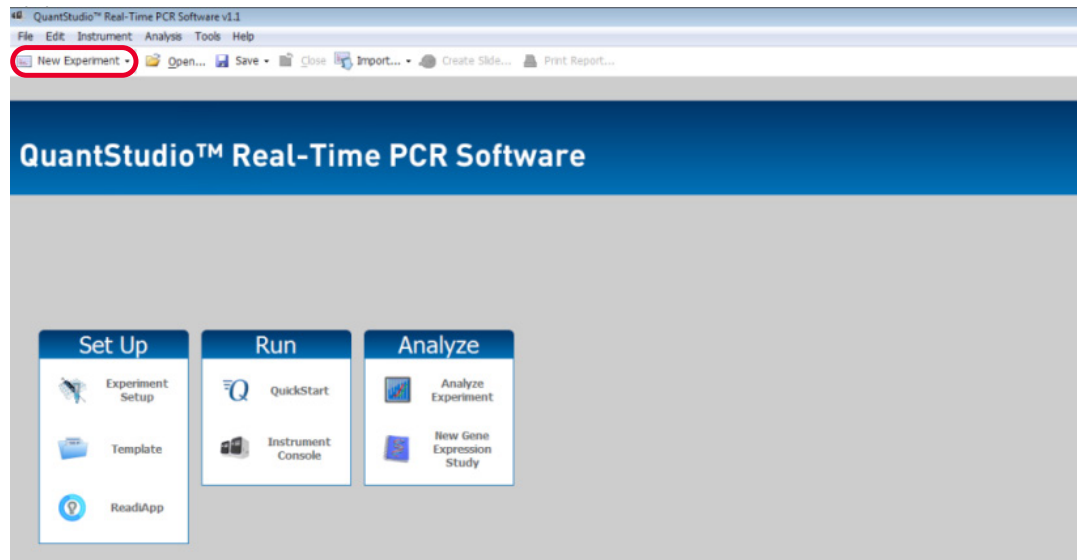
**Assay Performance:** It is expected that Cq values for positive controls and unknown samples should demonstrate amplification between cycles 10 and 26. Cq values determined for positive controls during internal validation are provided in Tables 2 and 4 for each respective kit as a guideline. Due to variations in instrument software versions, master mix preparations, pipetting, or DNA concentration, these values may shift but this does not invalidate the assay results.

## Streck ARM-D® Kits: Applied Biosystems QuantStudio 7 Flex Real-Time PCR System

Specific set-up instructions are provided for the Streck ARM-D Kit, *ampC* and Streck ARM-D Kit,  $\beta$ -Lactamase.

### Instrument Set-up

Open QuantStudio Real-Time PCR Software.



On the **Experiment Properties** tab in the Setup window select the following:

- Instrument type: QuantStudio 7 Flex System.
- Block: Fast 96-well.
- Type of experiment: Standard Curve.
- Reagents: TaqMan Reagents.
- Properties for the instrument run: Fast.

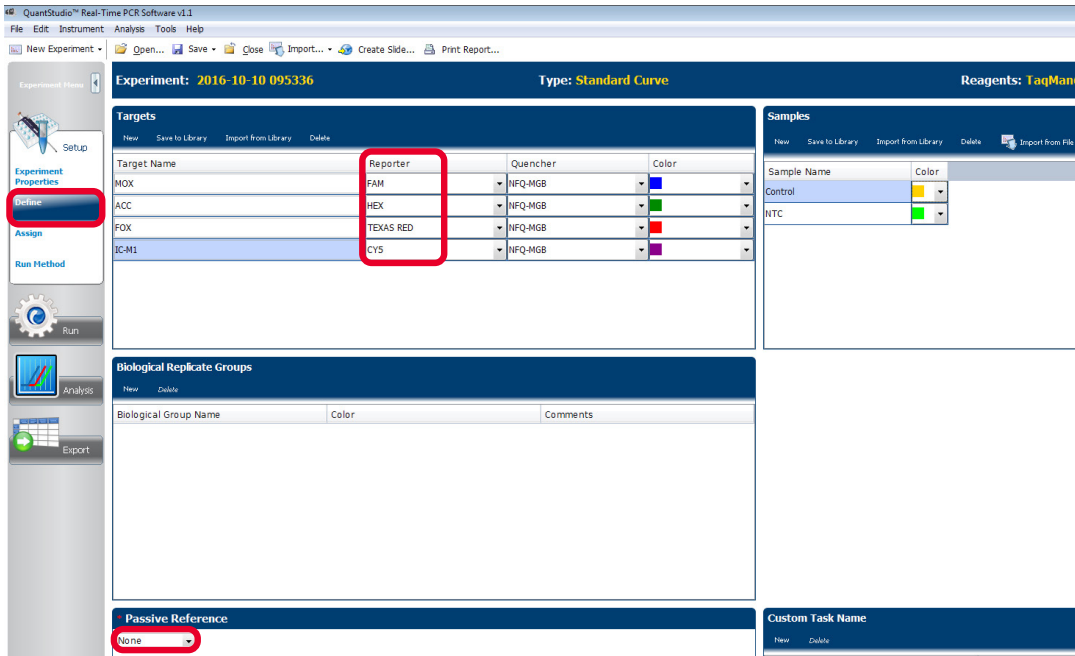
**Note:** The QuantStudio 7 Flex Real-Time PCR System is available with different block configurations. The Streck ARM-D Kits have been validated with the Fast 96-well block.

On the **Define** tab in the Setup window select the compatible reporter fluorophores needed to detect the targets in the Streck ARM-D Kits (i.e., FAM, HEX, Texas Red (equivalent to TEX615), and CY5 (equivalent to TYE665)).

**Note:** The QuantStudio 7 Flex Real-Time PCR System was calibrated for the fluorophores described above during validation of the Streck ARM-D Kits. If these dyes are not calibrated in the system, consult the instrument's manual to determine other equivalent reporters with similar excitation and emission spectra that might be selected for target detection.

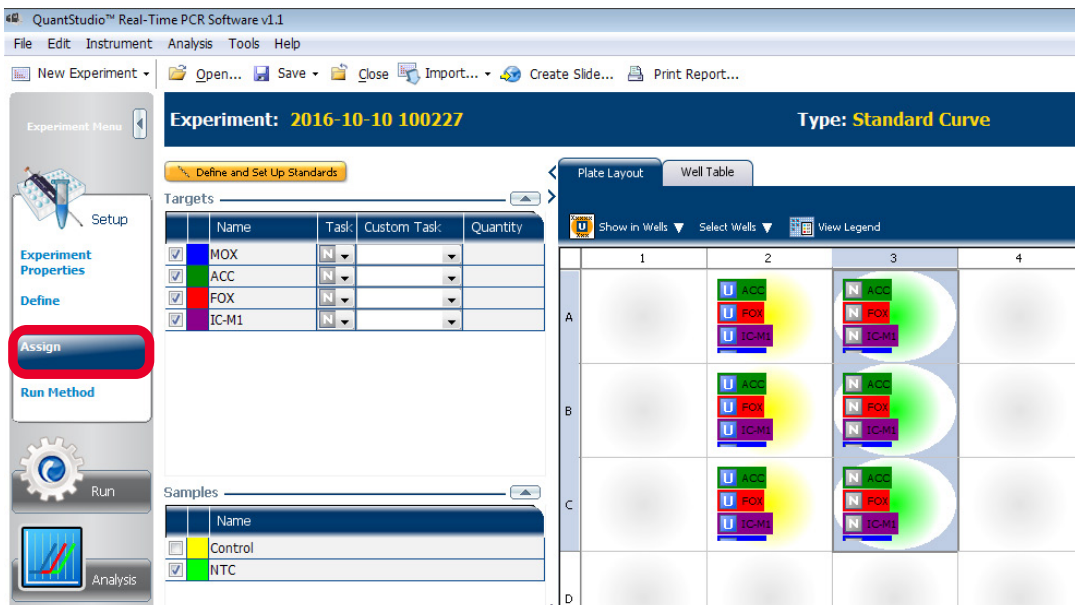
# Streck ARM-D® Kits: Applied Biosystems QuantStudio 7 Flex Real-Time PCR System

**Important:** Make sure that “None” is selected for the Passive Reference dye at the bottom of this window.



On the Assign tab in the Setup window select wells and assign the appropriate samples, targets, and tasks (Unknown or Negative Control). There should be no more than four targets in any single well.

The fluorophore/optical channel combinations for the targets covered in each PCR Mix are described in Table 1 (ARM-D Kit, *ampC*) and Table 3 (ARM-D Kit,  $\beta$ -Lactamase).



On the Run Method tab in the Setup window enter the Streck ARM-D Kit protocol (see example next page). Note that PCR cycling protocol is the same for both Streck ARM-D *ampC* and  $\beta$ -Lactamase Kits.

# Streck ARM-D® Kits: Applied Biosystems QuantStudio 7 Flex Real-Time PCR System

Streck ARM-D Kit Real-Time PCR Cycling Protocol	
Hot-start	98°C for 30 sec
30 cycles of	98°C for 5 sec
	60°C for 10 sec
	72°C for 20 sec (Detection Step)

**Important:** The following changes must also be made to the software default values:

Change Reaction Volume to 25 µL.

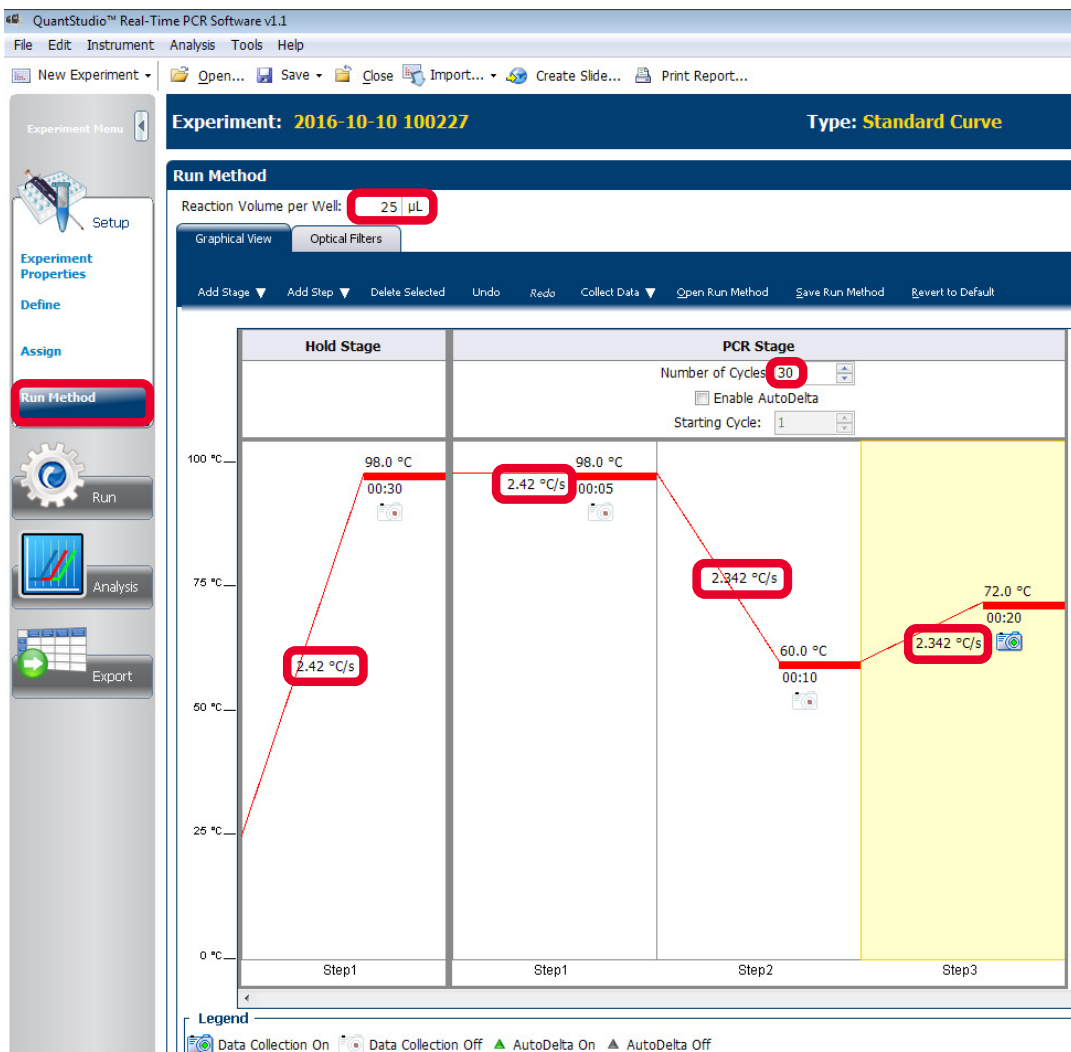
Change Number of Cycles in the PCR Stage to 30.

Change Ramp rates to these values:

2.42 °C/s for Step 1 in both the Hold Stage and PCR Stage.

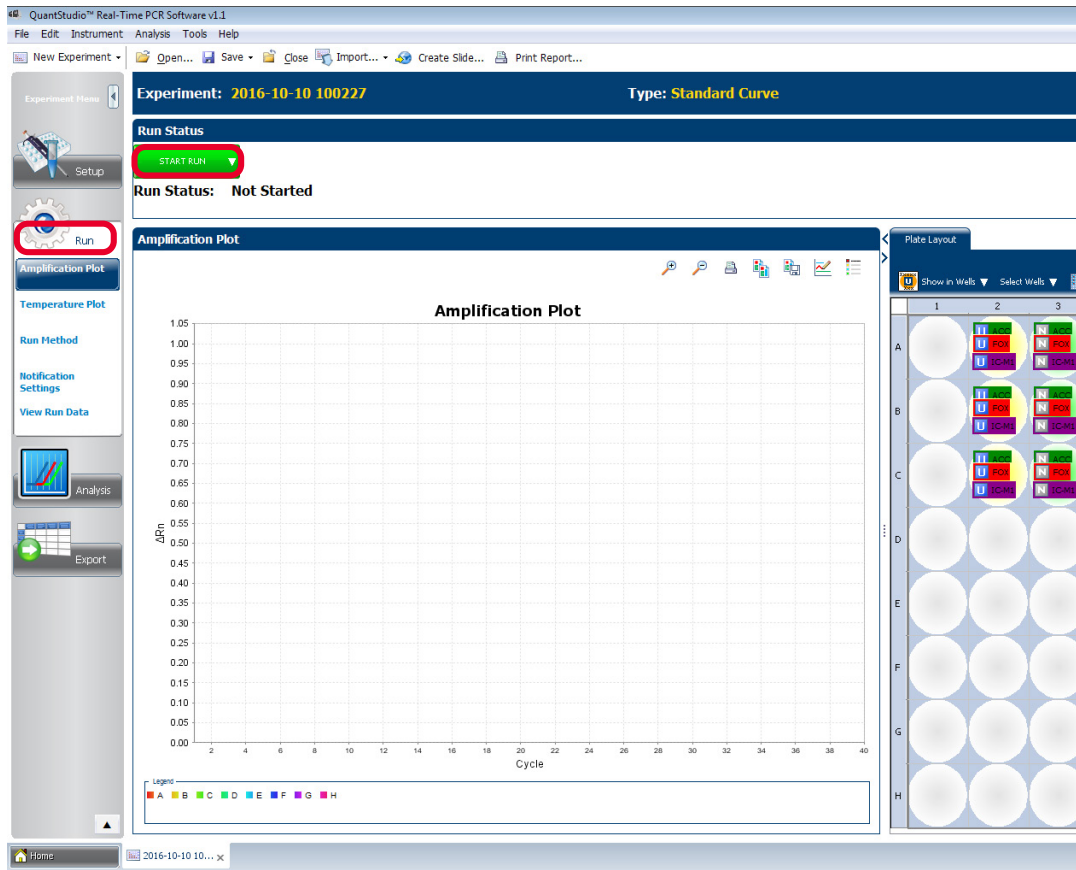
2.342 °C/s for Steps 2 and 3 in the PCR Stage.

Make sure Data Collection On is active after the extension step, as indicated by the camera icon.



# Streck ARM-D® Kits: Applied Biosystems QuantStudio 7 Flex Real-Time PCR System

On the Run window, click Start Run. The run should be complete within 45 minutes.



## Data Analysis and Data Interpretation: Streck ARM-D Kit, *ampC*

In order to interpret the data, each real-time PCR run must be verified with the Control Mix vials provided in the kit. Threshold cycle or quantification cycle values (Ct or Cq\*) for the positive controls should fall within the range recommended in the Instructions For Use. If the Cq values fall outside of the 10 to 26 cycle range, data should be interpreted carefully prior to confirming a positive or negative result. Expected positive control Cq values for each target are provided in Table 2. These values were determined during kit validation using the recommended Baseline/Threshold settings (Table 1) and are meant to be used as a point of reference. These values may change on a case-by-case basis.

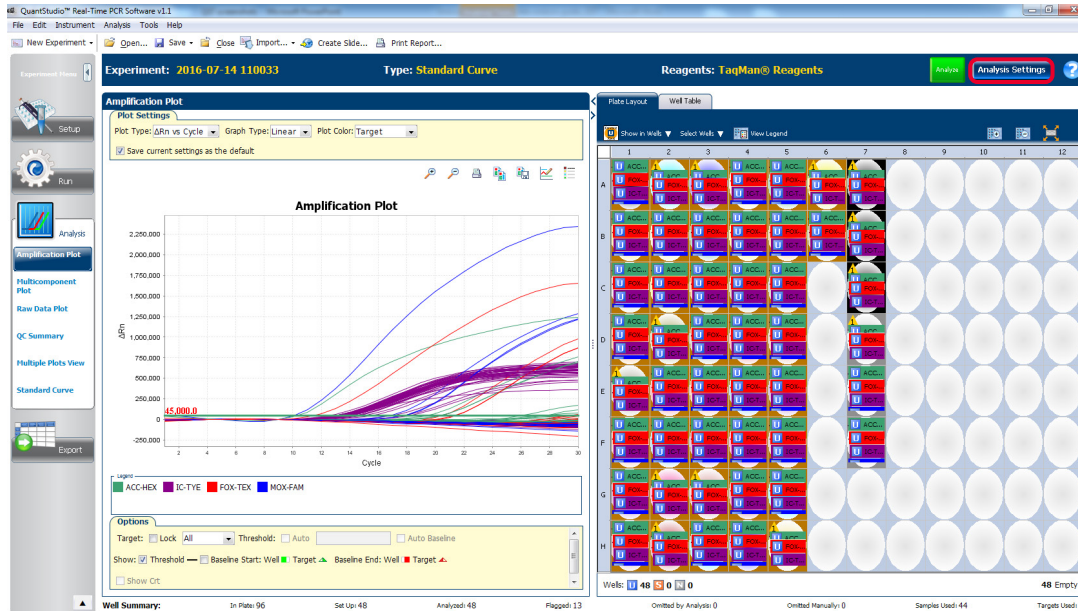
### Threshold values and baseline settings

Prior to analyzing Cq values in the opened data file, threshold values and baseline settings for each fluorophore should be manually set following guidelines described in Table 1.

\* For practical purposes, the Ct and Cq terms are used as synonyms in this guide. They represent the partial cycle number at which the fluorescence signal from a well and an optical channel exceeds the set threshold value.

# Streck ARM-D® Kits: Applied Biosystems QuantStudio 7 Flex Real-Time PCR System

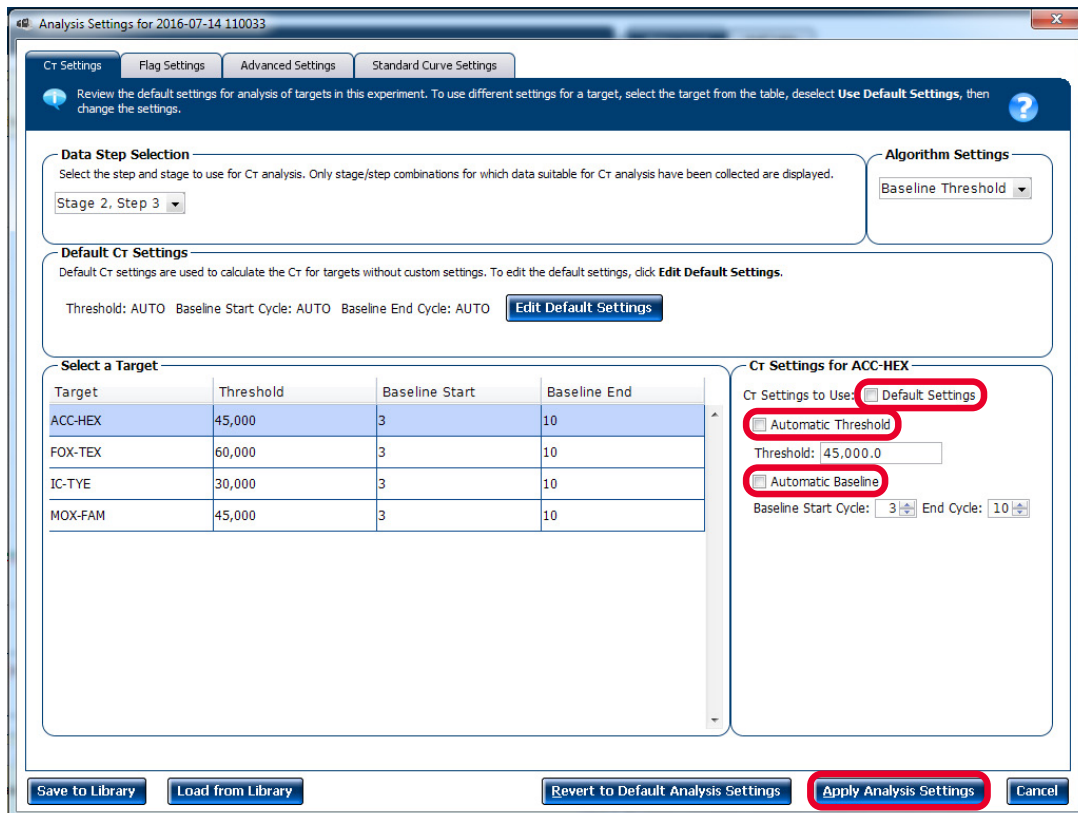
Click on the Analysis Settings button located at the top right hand corner of the analysis screen.



On the Analysis Settings window, deselect the following: Default Settings, Automatic Threshold, and Automatic Baseline.

Enter the corresponding threshold and baseline values specified in Table 1 (i.e., threshold value for FAM (MOX and DHA) and HEX (ACC and ACT/MIR) is 45,000; Baseline Start Cycle is 3 and End Cycle is 10). Repeat for each target/fluorophore combination.

Click Apply Analysis Settings button.



# Streck ARM-D® Kits: Applied Biosystems QuantStudio 7 Flex Real-Time PCR System

Table 1: Optical channels and threshold values determined during validation of the Streck ARM-D Kit, *ampC* on the QuantStudio 7 Flex Real-Time PCR System.

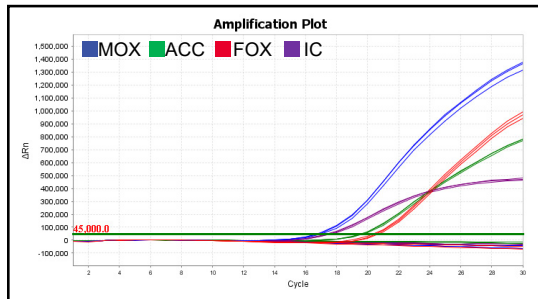
Master Mix	Target Gene Family	Fluorophore	Optical Channel	Threshold values	Baseline Starts at Cycle	Baseline ends at Cycle
PCR Mix 1	MOX	FAM	FAM	45,000	3	10
	ACC	HEX	HEX	45,000	3	10
	FOX	TEX615	Texas Red	60,000	3	10
	IC	TYE665	Cy5	30,000	3	10
PCR Mix 2	DHA	FAM	FAM	45,000	3	10
	ACT/MIR (EBC)	HEX	HEX	45,000	3	10
	CMY-2	TEX615	Texas Red	60,000	3	10
	IC	TYE665	Cy5	30,000	3	10

## Amplification Curve Data

After setting threshold and baseline values, all PCR amplification curves should be visually inspected to confirm amplification of the sample and that optimal baseline and threshold settings are set for analysis of the data. Characteristic amplification data for positive control targets of Streck ARM-D Kit, *ampC* is shown in Figure 1. Although Cq values for amplification plots of unknown samples may vary from sample to sample, representative amplification data of plasmid-mediated *ampC*-positive clinical isolates is shown in Figure 2.

Refer to the **Data Interpretation** section at the end of this document for specific guidelines on interpreting unknown sample data.

### Control Mix 1



### Control Mix 2

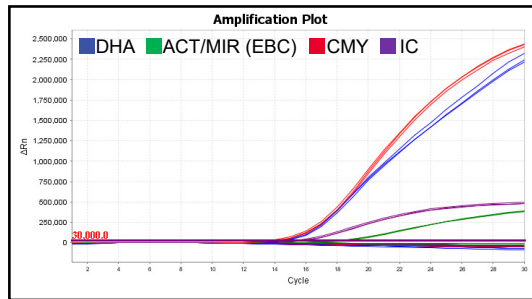
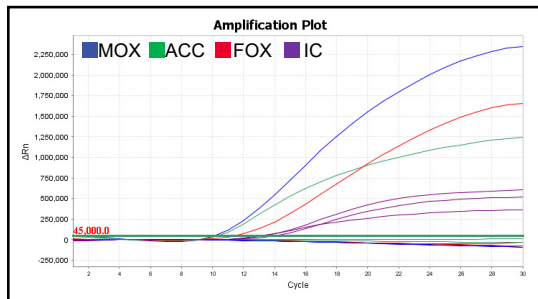


Figure 1: Multiplex real-time PCR amplification data of positive DNA Control Mixes (n=3) for the Streck ARM-D Kit, *ampC*, on the QuantStudio 7 Flex Real-Time PCR System.

### PCR Mix 1



### PCR Mix 2

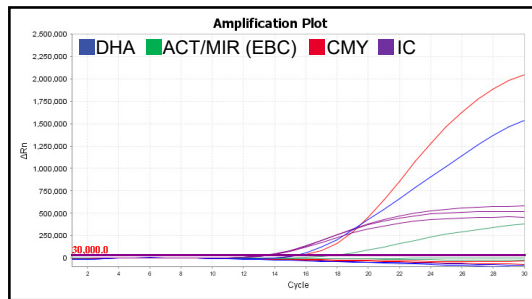


Figure 2: Amplification of plasmid-mediated *ampC*-positive clinical isolates using Streck ARM-D Kit, *ampC*. The data above shows amplification of six clinical isolates that are positive for each respective *ampC* target detected by the kit. The internal control (IC, indicated by purple lines) was detected in each sample.

## Streck ARM-D® Kits: Applied Biosystems QuantStudio 7 Flex Real-Time PCR System

### Cq Values – Controls

When setting threshold and baseline values for each target as specified in Table 1, Cq values obtained for positive controls during kit validation on the QuantStudio 7 Flex Real-Time PCR System fell within the range specified in Table 2. These values should serve as a point of reference for typical results. However, different software versions, master mix preparations, or variation in the DNA concentration may produce different values. Deviation from the values below does not invalidate the assay. Contact Streck Technical Services for help with interpretation if necessary.

**Table 2:** Cq values for positive control targets determined during validation of the Streck ARM-D Kit, *ampC*.

Control Mix	Target Gene Family (Fluorophore)	Cq Value Range
Mix 1	MOX (FAM)	17 ± 3
	ACC (HEX)	20 ± 3
	FOX (TEX615)	21 ± 3
	IC (TYE665)	17 ± 3
Mix 2	DHA (FAM)	16 ± 3
	ACT/MIR (HEX)	20 ± 3
	CMY-2 (TEX615)	16 ± 3
	IC (TYE665)	17 ± 3

### Cq Values – Unknown Samples

To classify unknown samples as positive or negative for *ampC* targets, refer to the **Data Interpretation** section for specific guidelines on interpreting sample.

## Data Analysis and Data Interpretation: Streck ARM-D Kit, β-Lactamase

In order to interpret the data, each real-time PCR run must be verified with the Control Mix vials provided in the kit. Threshold cycle or quantification cycle values (Ct or Cq\*\*) for the positive controls should fall within the range recommended in the Instructions For Use. If the Cq values fall outside of the 10 to 26 cycle range, data should be interpreted carefully prior to confirming a positive or negative result. Expected positive control Cq values for each target are provided in Table 4. These values were determined during kit validation using the recommended Baseline/Threshold settings (Table 3) and are meant to be used as a point of reference. These values may change on a case-by-case basis.

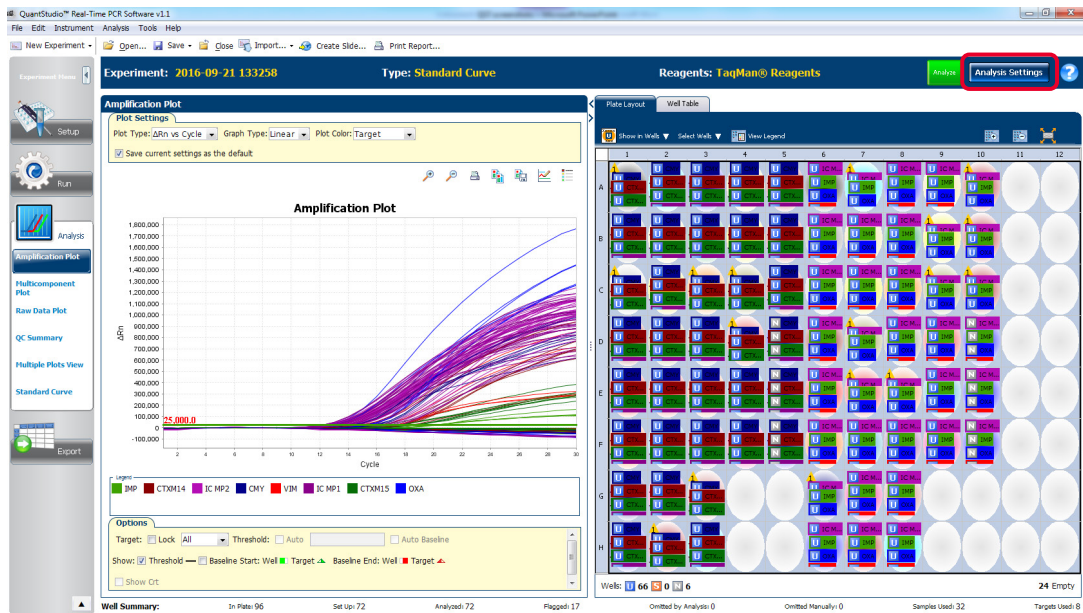
### Threshold values

Prior to analyzing Cq values in the opened data file, threshold values and baseline settings for each fluorophore must be manually set following guidelines described in Table 3.

Click on the **Analysis Settings** button located at the top right hand corner of the analysis screen.

\*\* For practical purposes, the Ct and Cq terms are used as synonyms in this guide. They represent the partial cycle number at which the fluorescence signal from a well and an optical channel exceeds the set threshold value.

# Streck ARM-D® Kits: Applied Biosystems QuantStudio 7 Flex Real-Time PCR System



On the Analysis Settings window, deselect the following: Default Settings, Automatic Threshold, and Automatic Baseline.

Enter the corresponding threshold and baseline values specified in Table 1 (i.e., threshold value for FAM (CMY, OXA-48, and DHA) is 30,000; Baseline Start Cycle is 3 and End Cycle is 10). Repeat for each target/fluorophore combination.

Click Apply Analysis Settings button.

The screenshot shows the 'Analysis Settings for 2016-09-21 133258' dialog box. The 'Cr Settings' tab is selected. It contains several sections: 'Data Step Selection' (Stage 2, Step 3), 'Default Cr Settings' (Threshold: AUTO, Baseline Start Cycle: AUTO, Baseline End Cycle: AUTO), 'Select a Target' (a table with columns for Target, Threshold, Baseline Start, and Baseline End), and 'Cr Settings for CMY' (with options for Default Settings, Automatic Threshold, and Automatic Baseline). The 'Apply Analysis Settings' button is highlighted with a red circle.

Target	Threshold	Baseline Start	Baseline End
CMY	30,000	3	10
CTXM14	45,000	3	10
CTXM15	25,000	3	10
IC MP1	40,000	3	10
IC MP2	40,000	3	10
IMP	25,000	3	10
OXA	30,000	3	10
VIM	45,000	3	10

## Streck ARM-D® Kits: Applied Biosystems QuantStudio 7 Flex Real-Time PCR System

**Table 3:** Optical channels and threshold values determined during validation of the Streck ARM-D Kit,  $\beta$ -Lactamase on the QuantStudio 7 Flex Real-Time PCR System.

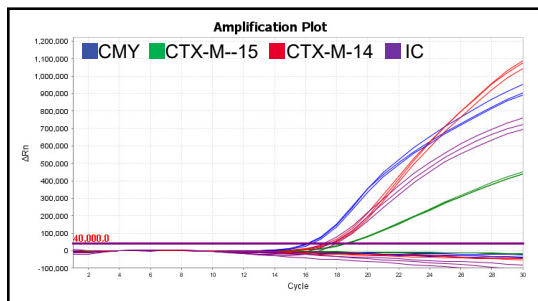
Master Mix	Target Gene Family	Fluorophore	Optical Channel	Threshold Values	Baseline Starts at Cycle	Baseline Ends at Cycle
PCR Mix 1	CMY-2	FAM	FAM	30,000	3	10
	CTX-M-15	HEX	HEX	25,000	3	10
	CTX-M-14	TEX615	Texas Red	45,000	3	10
	IC	TYE665	Cy5	40,000	3	10
PCR Mix 2	OXA-48	FAM	FAM	40,000	3	10
	IMP	HEX	HEX	40,000	3	10
	VIM	TEX615	Texas Red	45,000	3	10
	IC	TYE665	Cy5	40,000	3	10
PCR Mix 3	DHA	FAM	FAM	30,000	3	10
	KPC	HEX	HEX	25,000	3	10
	NDM	TEX615	Texas Red	45,000	3	10
	IC	TYE665	Cy5	40,000	3	10

### Amplification Curve Data

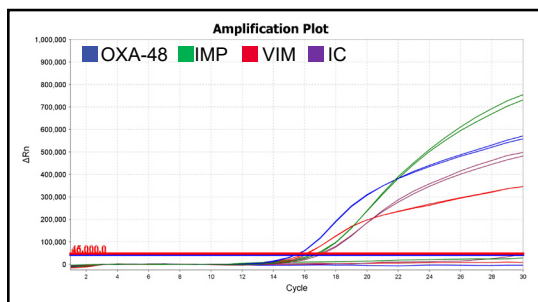
After setting threshold and baseline values, all PCR amplification curves should be visually inspected to confirm proper amplification.

Characteristic amplification data for positive control targets detected with Streck ARM-D Kit,  $\beta$ -Lactamase is shown in Figure 3. Although Cq values for amplification plots of unknown samples may vary from sample to sample, representative amplification data of  $\beta$ -lactamase-positive clinical isolates is shown in Figure 4. Refer to the **Data Interpretation** section at the end of this document for specific guidelines on interpreting unknown sample data.

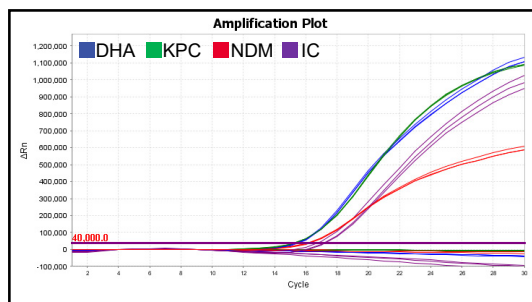
### Control Mix 1



### Control Mix 2

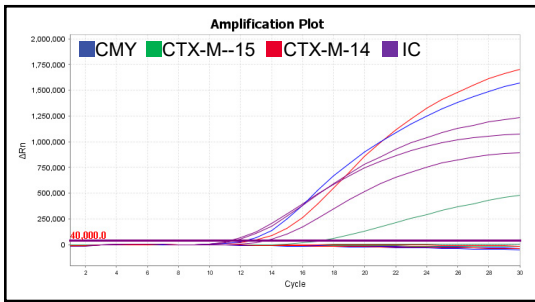


### Control Mix 3

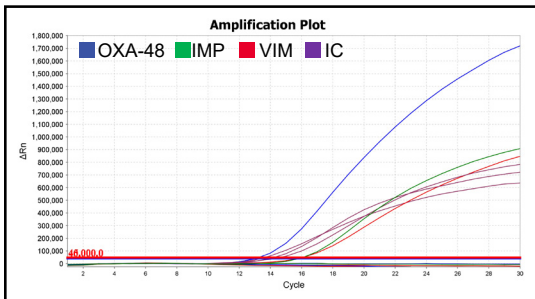


*Figure 3: Multiplex real-time PCR amplification data of positive DNA Control Mixes (n=3) of Streck ARM-D Kit,  $\beta$ -Lactamase on the QuantStudio 7 Flex Real-Time PCR System.*

PCR Mix 1



PCR Mix 2



PCR Mix 3

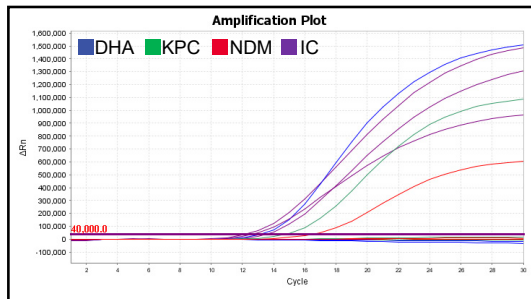


Figure 4: Amplification of  $\beta$ -lactamase-positive clinical isolates using Streck ARM-D Kit,  $\beta$ -Lactamase. Data shows the amplification of nine DNA samples that are positive for one of the respective  $\beta$ -Lactamase targets detected with the kit on the QuantStudio 7 Flex Real-Time PCR System. The internal control (IC, indicated by purple lines) was detected in each sample.

Cq Values – Controls

When setting threshold and baseline values for each target as specified in Table 3, Cq values obtained for positive controls during kit validation on the QuantStudio 7 Flex Real-Time PCR System fell within the range specified in Table 4. These values should serve as a point of reference for typical results. However, different software versions, master mix preparations, or variation in the DNA concentration may produce different values. Deviation from the values below does not invalidate the assay. Contact Streck Technical Services for help with interpretation if necessary.

## Streck ARM-D® Kits: Applied Biosystems QuantStudio 7 Flex Real-Time PCR System

**Table 4:** Cq values for positive control targets determined during validation of the Streck ARM-D Kit,  $\beta$ -Lactamase on the QuantStudio 7 Flex Real-Time PCR System.

Control Mix	Target Gene Family (Fluorophore)	Cq Value Range
Control Mix 1	CMY-2 (FAM)	16 ± 3
	CTX-M-15 (HEX)	18 ± 3
	CTX-M-14 (TEX615)	17 ± 3
	IC (TYE665)	17 ± 3
Control Mix 2	OXA-48 (FAM)	14 ± 3
	IMP (HEX)	16 ± 3
	VIM (TEX615)	15 ± 3
	IC (TYE665)	15 ± 3
Control Mix 3	DHA (FAM)	15 ± 3
	KPC (HEX)	15 ± 3
	NDM (TEX615)	16 ± 3
	IC (TYE665)	17 ± 3

### Cq Values – Unknown samples

To classify unknown samples as positive or negative for  $\beta$ -lactamase targets, refer to Data Interpretation section for specific guidelines on interpreting sample data.

## Data Interpretation: Unknown Samples

### Cq values and data interpretation of unknown samples with Streck ARM-D Kits

To classify unknown samples as positive or negative for the respective  $\beta$ -lactamase targets, Cq values specified in Table 5 should be followed as a guideline, taking into account that Cq values of unknown samples will vary depending on the starting DNA concentration.

**Table 5:** Data interpretation for unknown samples.

Measured Cq FAM, HEX, TEX615	Cq IC (TYE665)	Interpretation
≤ 26*	10-20*	Positive Sample
NA	10-20*	Negative Sample
NA or > 26	NA or > 26	Invalid

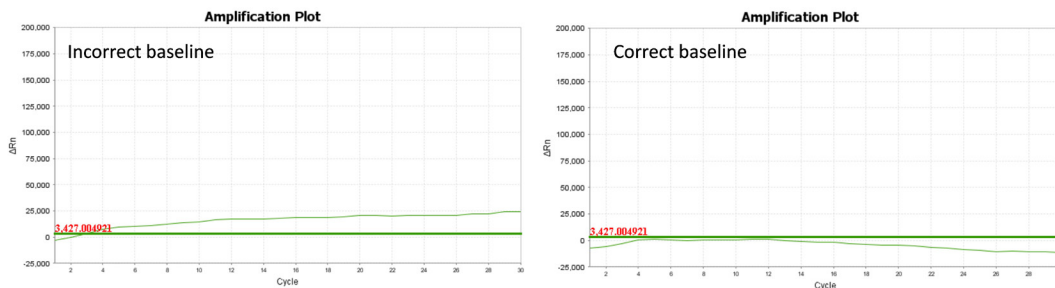
\* Typical Cq values obtained for 10-200 ng/ $\mu$ L purified DNA samples.

**Positive Sample:** Overall, unknown samples (using 10-200 ng/ $\mu$ L DNA in a PCR) may be interpreted as positive if the Cq value is ≤ 26 cycles.

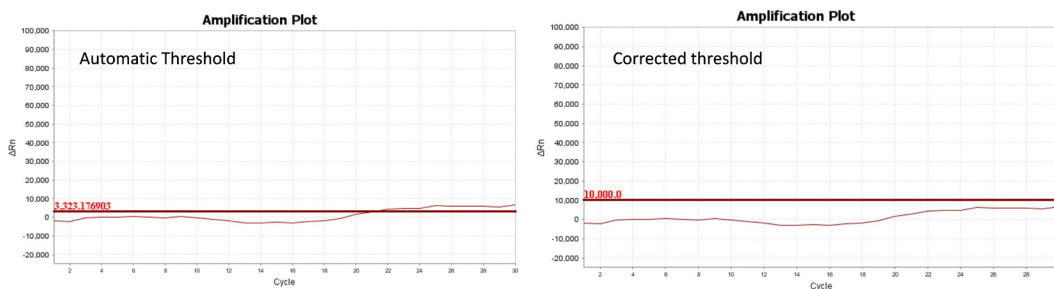
**Negative Sample:** If no Cq value is detected in the FAM, HEX, and Texas Red channels for unknown samples, confirm the sample was added to the reactions by verifying positive amplification of the internal control (IC) for Gram-negative bacteria in the Cy5 channel (Cq= 10 - 20). If IC (Cy5) is amplified and no amplification is detected in FAM, HEX, and Texas Red channels, the unknown sample may be interpreted as negative for the respective resistance mechanisms appropriate for each probe provided within the kit.

## Troubleshooting

- 1. Ramp rate error message:** If the instrument's default ramp rates with a Fast 96-well block are not changed as described in the Instrument Setup instructions in these guidelines, an error message window will be displayed just before starting the PCR run. Before proceeding with the run, adjust the ramp rates at each stage of the PCR Protocol, in the Run Method window, as previously described in the instrument setup instructions.
- 2. Amplification is not observed for any sample after the PCR protocol is complete:** Verify that ROX is not selected as a passive reference dye on the Experiment Setup window. Refer to instrument setup instructions at the beginning of this document for verification that the passive dye selected is "None". If amplification is still not observed after the correction or if "None" was already selected, the PCR run must be repeated.
- 3. Exponential Algorithm Fail (EXPFAIL) Flag on unknown samples:** It is not uncommon that after data processing by the QuantStudio Real-Time PCR Software, some unknown samples will display an EXPFAIL Flag on the QC Summary window. As described by the instrument manufacturer, the error message indicates failure to identify the exponential phase of the amplification plot for that particular sample. PCR amplification curves of these samples must be carefully inspected in order to confirm proper amplification prior to evaluation of Cq values determined by the software. Data can be interpreted if proper amplification curve data is obtained for these samples.
- 4. Incorrect baseline settings:** For some samples, automatic baseline settings that are erroneously assigned may cause false positive or false negative values. In the example shown below, the automatic baseline calculation identifies a negative sample as a positive sample (left image) although there is no PCR curve. This can be corrected by specifying baseline start and end cycles (right image).



- 5. False amplification due to incorrect threshold:** On occasion, some apparent increase in fluorescence that is not caused by target amplification may exceed threshold levels to result in a Cq value and therefore a false positive result. It is important to visually inspect the amplification curves for each well and target to detect potentially erroneous results. For example, in the Amplification graph below (left image) signal noise resulted in a Cq value when automatic threshold was set, indicating the potential presence of the specific target. However, on examination of the data, it is apparent that the increase in fluorescence intensity is not due to amplification, and the sample should be considered negative for that target. In this case, a higher threshold (set at 10,000) would have resulted in the correct interpretation (right image).



Refer to the Streck ARM-D Kit Frequently Asked Questions document for additional troubleshooting help or contact Streck Technical Services at 800.843.0912 or [technicalservices@streck.com](mailto:technicalservices@streck.com).