Streck ARM-D® Kits

Data Acquisition and Analysis Guide

Real-Time PCR Platform:
QIAGEN Rotor-Gene Q 5plex System

Quick Links

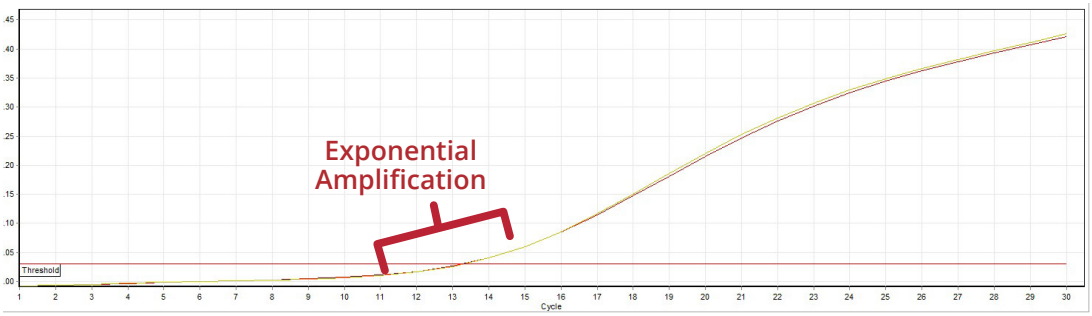
This guide is intended to be used as an ARM-D Kit-specific supplement for the Instructions For Use (IFU) document included with each kit. The Streck ARM-D kits referenced in this document are labeled as CE IVD and are For Export Only. Not for sale in the U.S. The instructions provided in this guide serve 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

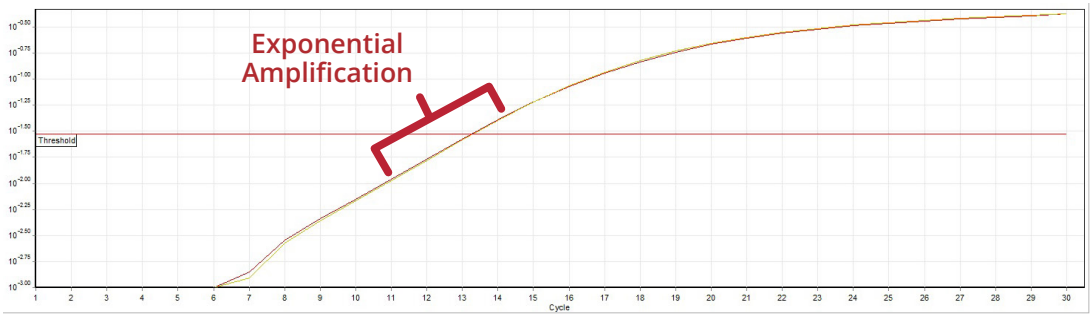
Instrument and Protocol Set-up: A template protocol can be saved and reused for future assays to reduce instrument set-up time after the first run with each kit.

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/µL 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.

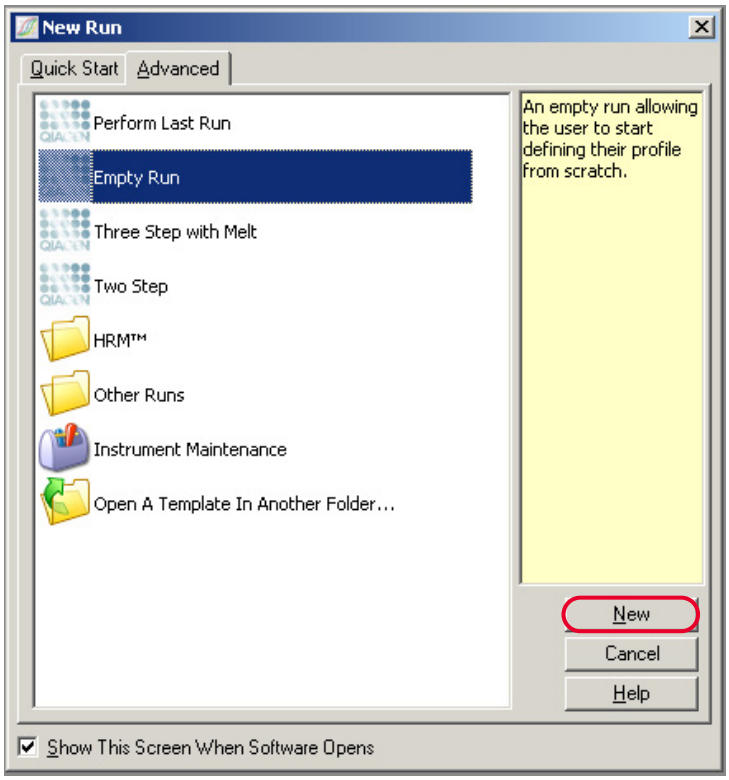
Specific set-up instructions are provided for the Streck ARM-D Kit, *ampC* and Streck ARM-D Kit, β-Lactamase.

Instrument Set-up

Open Rotor-Gene Q Series Software.

In the New Run window, click on the **Advanced** tab and then select **Empty Run**.

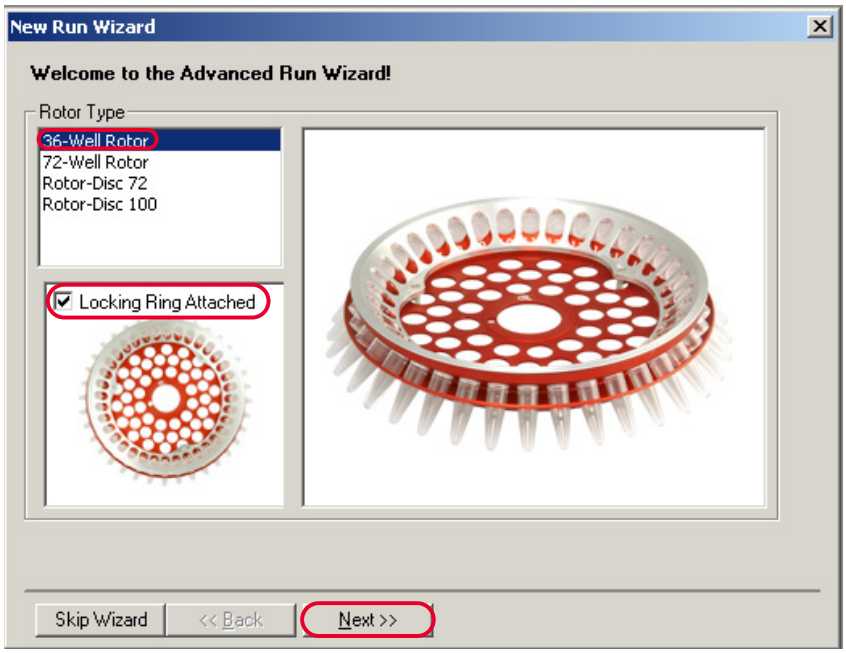
Click **New**.



In the New Run Wizard window select the appropriate **Rotor Type** installed on the instrument.

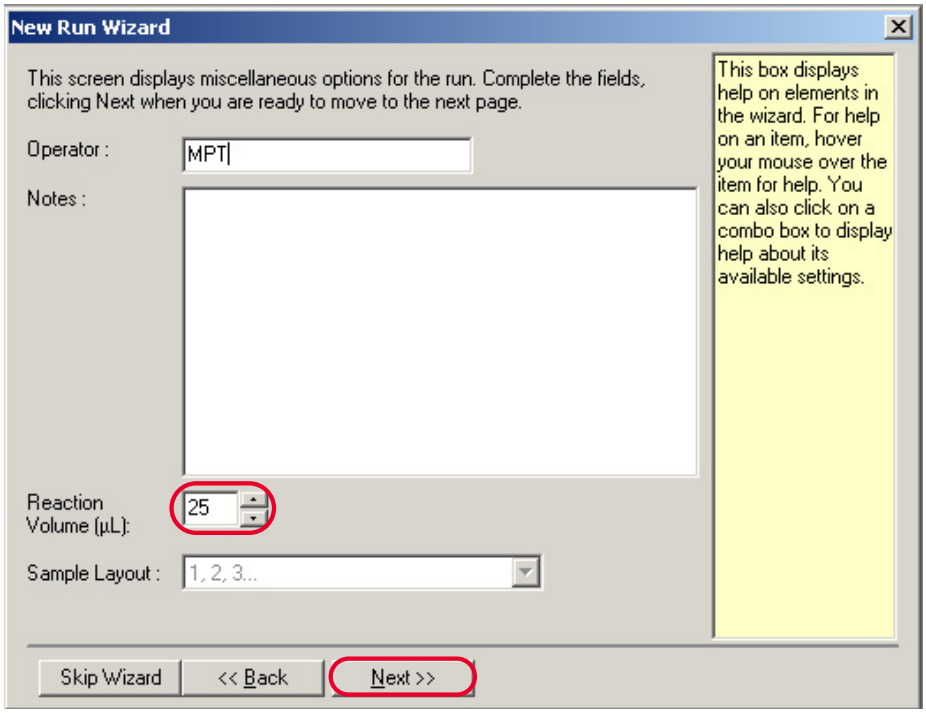
Select the check box next to Locking Ring Attached.

Click **Next**.

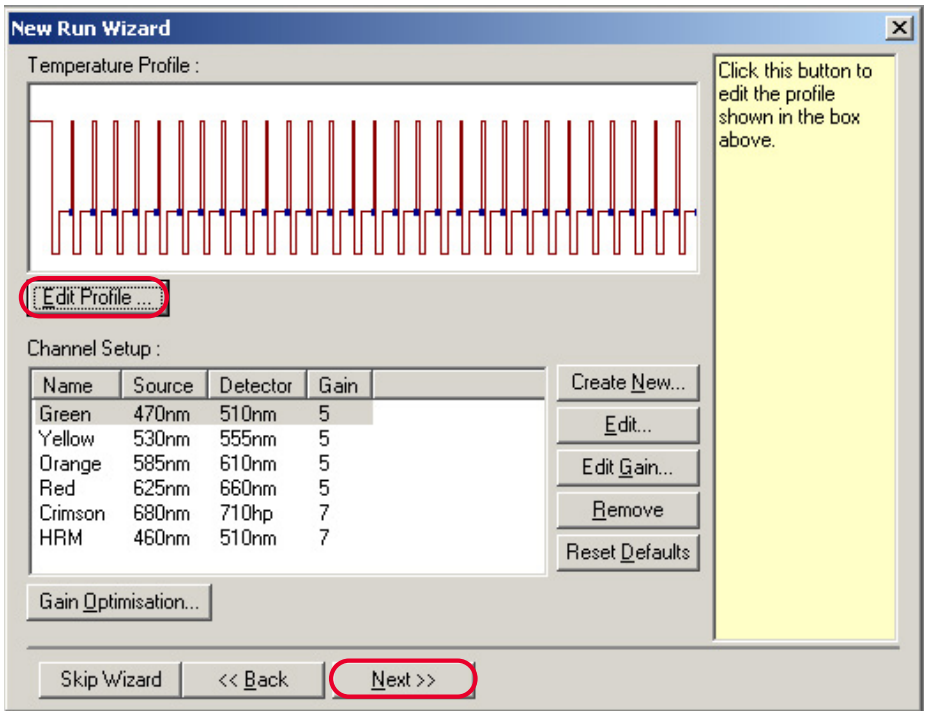


On the next screen enter 25 µL for the reaction volume.

Click **Next**.



In the New Run Wizard window for Temperature Profile click **Edit Profile**.



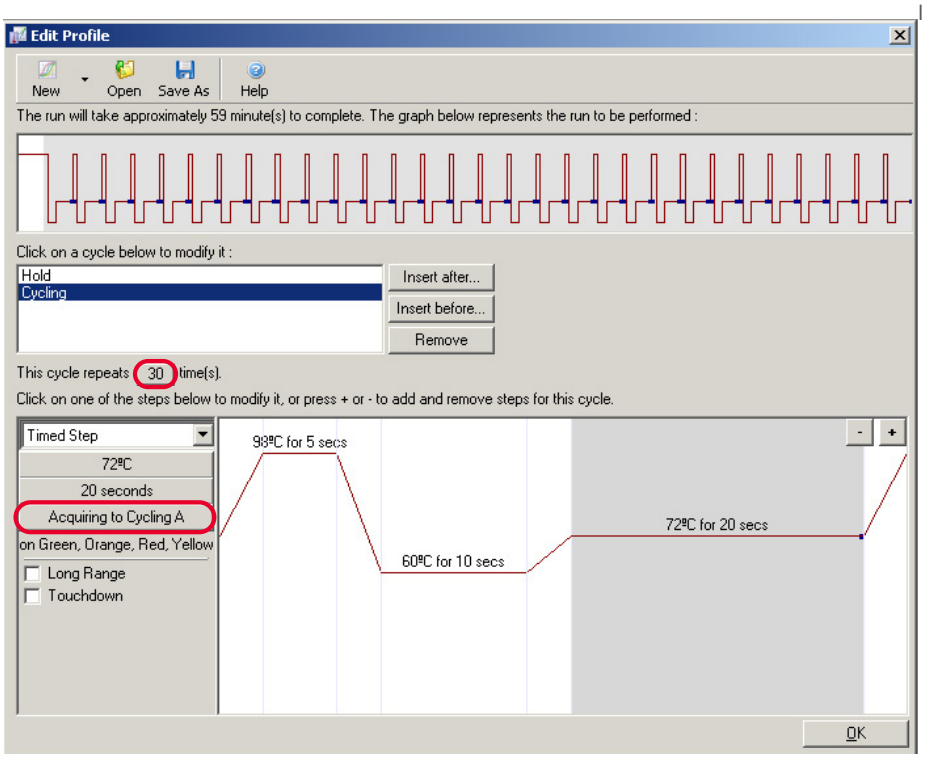
In the Edit Profile window, enter the Streck ARM-D Kit protocol as shown below. Note the PCR cycling protocol is the same for both Streck ARM-D *ampC* and β-Lactamase Kits.

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 number of cycle repeats to **30** times.

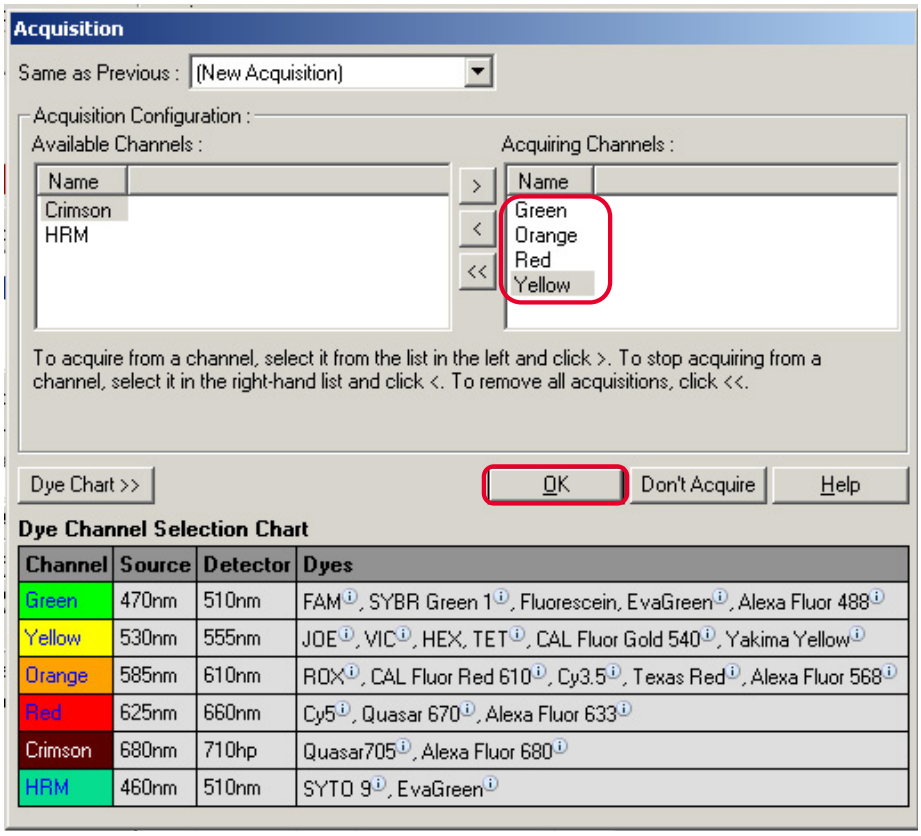
Make sure data is being collected on the optical channels needed to detect targets covered by the Streck ARM-D Kits by clicking on **Acquiring to Cycling A** in the extension step, as indicated below.



In the Acquisition window add the Acquiring Channels to detect targets for the Streck ARM-D Kits:

Green Channel detects FAM.
Yellow Channel detects HEX.
Orange Channel detects TEX615.
Red Channel detects TYE665.

Click **OK** on the Acquisition window.

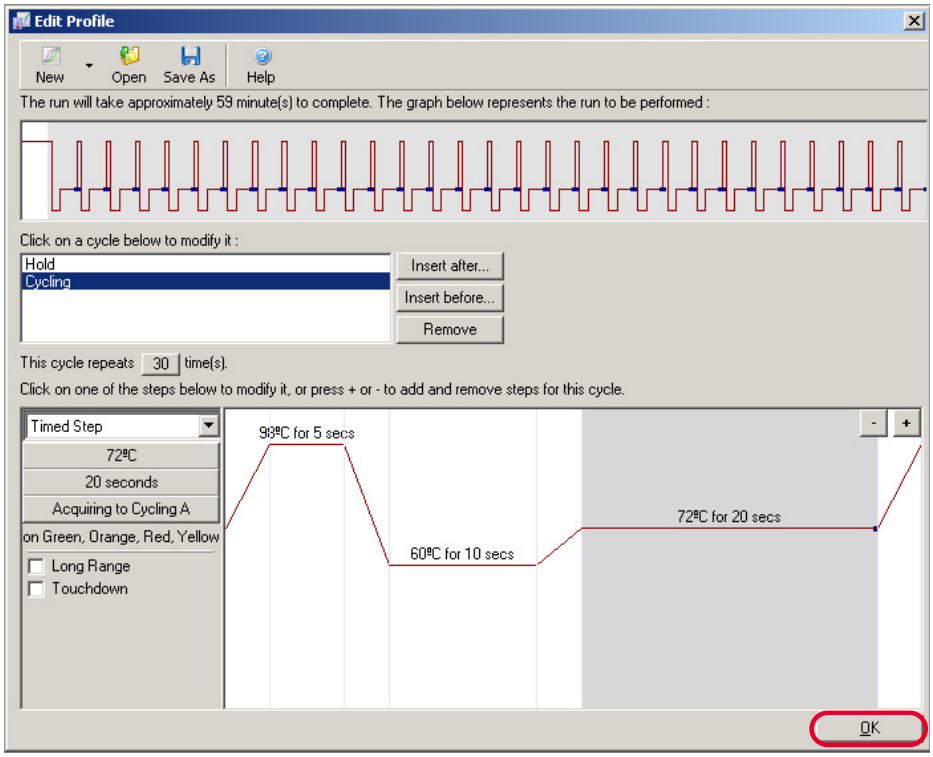


Dye Channel Selection Chart

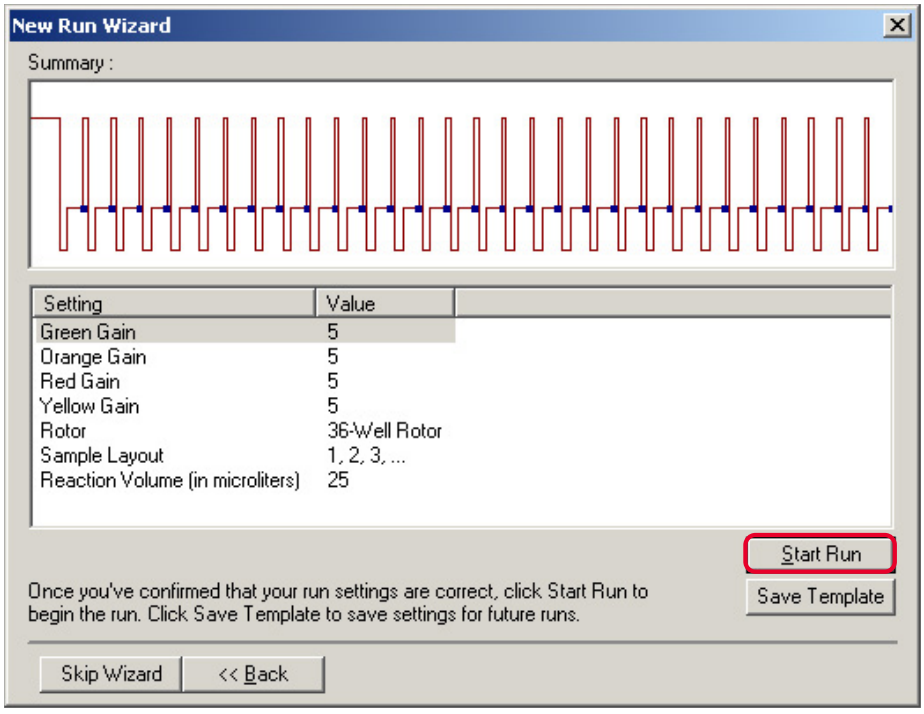
Channel	Source	Detector	Dyes
Green	470nm	510nm	FAM, SYBR Green 1, Fluorescein, EvaGreen, Alexa Fluor 488
Yellow	530nm	555nm	JOE, VIC, HEX, TET, CAL Fluor Gold 540, Yakima Yellow
Orange	585nm	610nm	ROX, CAL Fluor Red 610, Cy3.5, Texas Red, Alexa Fluor 568
Red	625nm	660nm	Cy5, Quasar 670, Alexa Fluor 633
Crimson	680nm	710hp	Quasar705, Alexa Fluor 680
HRM	460nm	510nm	SYTO 9, EvaGreen

Click the **Save As** button to save the protocol.

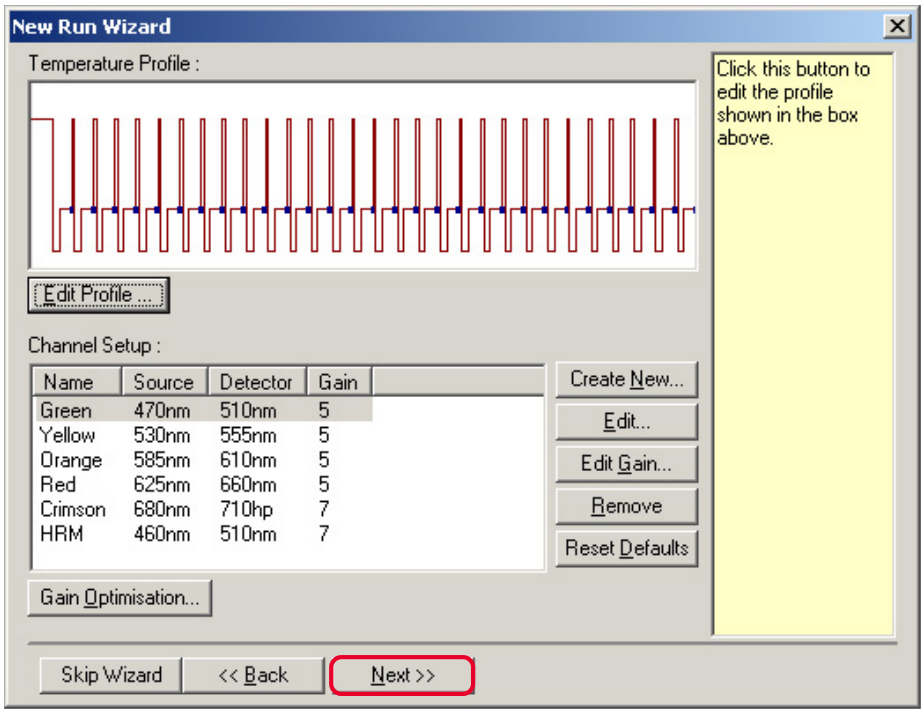
Click **OK** on the Edit Profile window.



Click **Next** on the New Run Wizard screen.

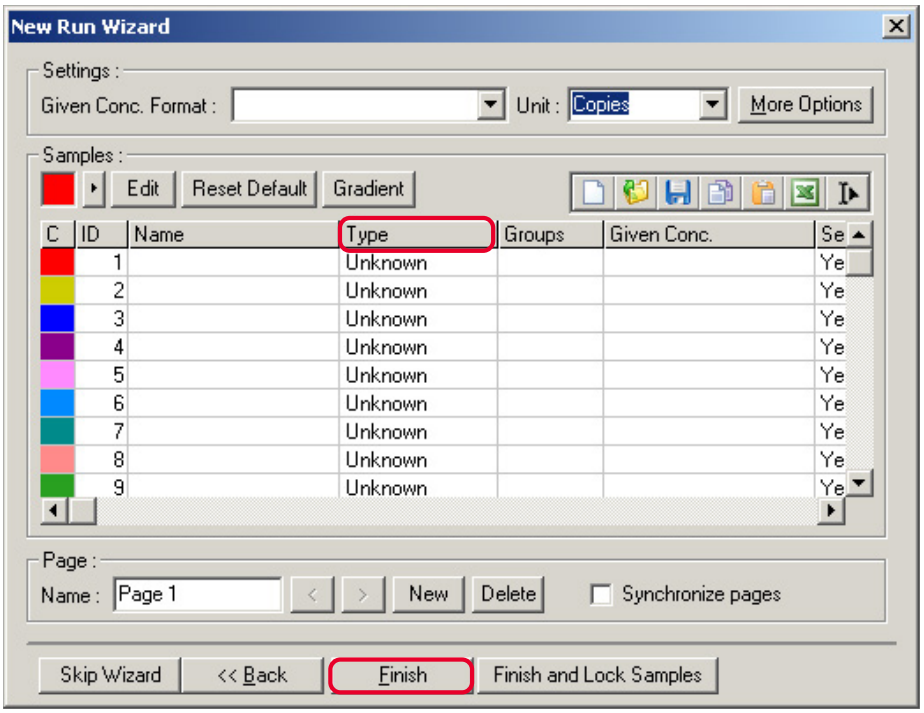


Click **Start Run**. The run should be complete within one hour.



Once PCR run starts, the samples types (**Unknown, Positive Control, and Negative Control**) and sample names can be identified in the New Run Wizard window.

Click **Finish.**



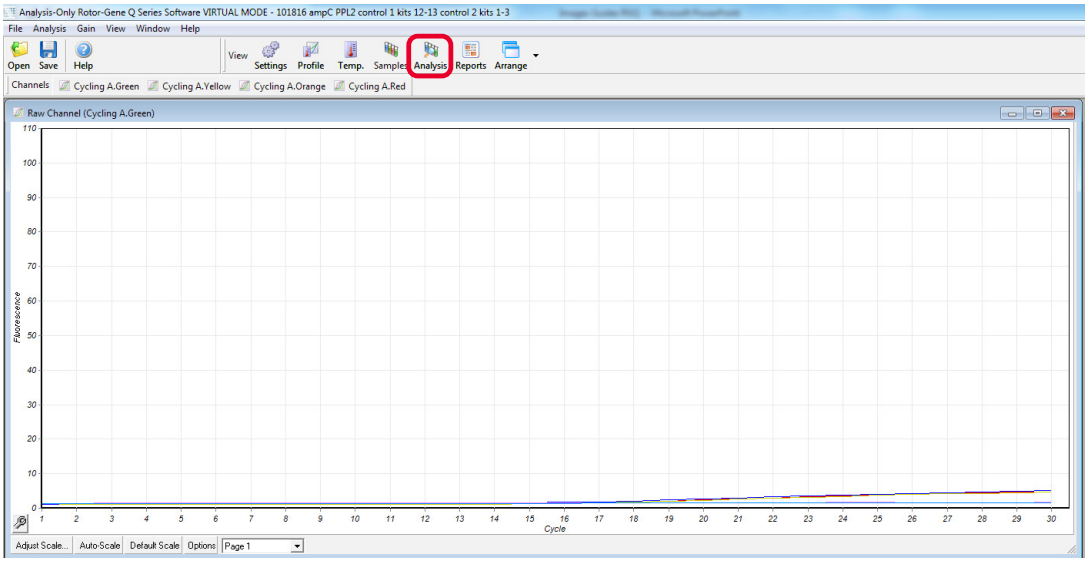
Data Analysis and Data Interpretation: Streck ARM-D Kit, *ampC*

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 for each fluorophore should be manually set following guidelines described in Table 1.

Click on the **Analysis** icon.



On the Analysis screen, select any of the four optical channels.

**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.*

Click **Show**.


Analysis
2 Std Curves (Rel.)
Other...
Quantitation
Melt
Cycling A.Green (Page 1)
Cycling A.Orange (Page 1)
Cycling A.Red (Page 1)
Cycling A.Yellow (Page 1)
Show
Hide
Auto-shrink window

Enter the corresponding threshold value for the optical channel as it is specified in Table 1 (i.e., threshold value for FAM-Green Channel is 0.05).

Select the Slope Correct button (Note that Dynamic Tube button is selected by default).

Verify the baseline calculations look acceptable for the optical channel.

Repeat procedure for each optical channel.

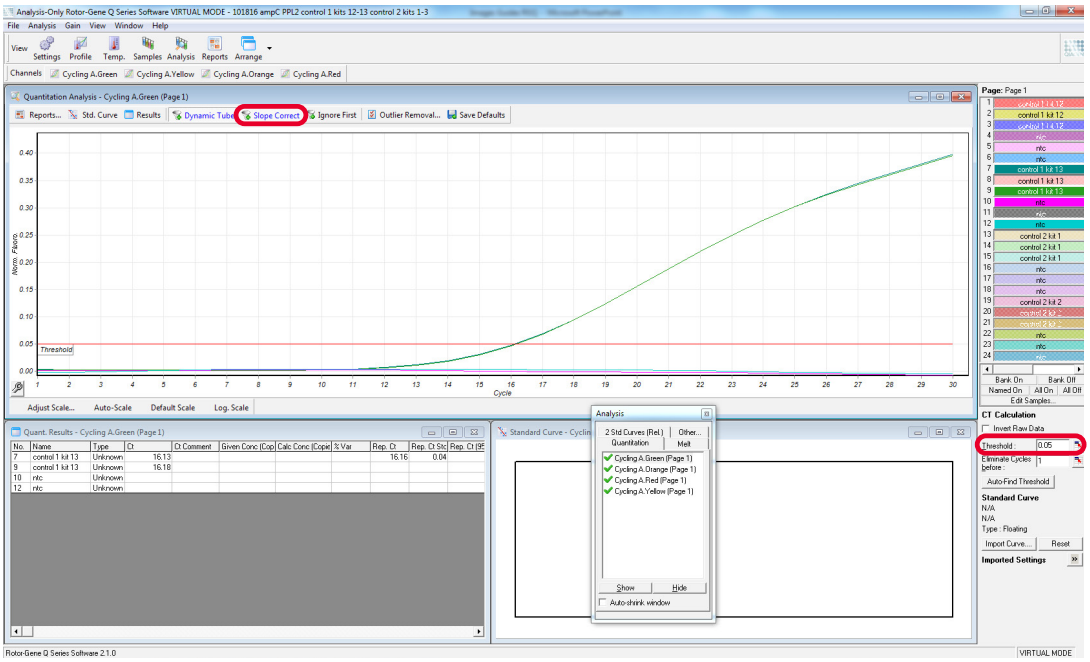


Table 1: Optical channels and threshold values determined during validation of the Streck ARM-D Kit, *ampC* on the QIAGEN Rotor-Gene Q 5Plex System.

Master Mix	Target Gene Family	Fluorophore	Optical Channel	Threshold Values
PCR Mix 1	MOX	FAM	Green	0.05
	ACC	HEX	Yellow	0.06
	FOX	TEX615	Orange	0.07
	IC	TYE665	Red	0.05
PCR Mix 2	DHA	FAM	Green	0.05
	EBC	HEX	Yellow	0.06
	CMY-2	TEX615	Orange	0.07
	IC	TYE665	Red	0.05

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.

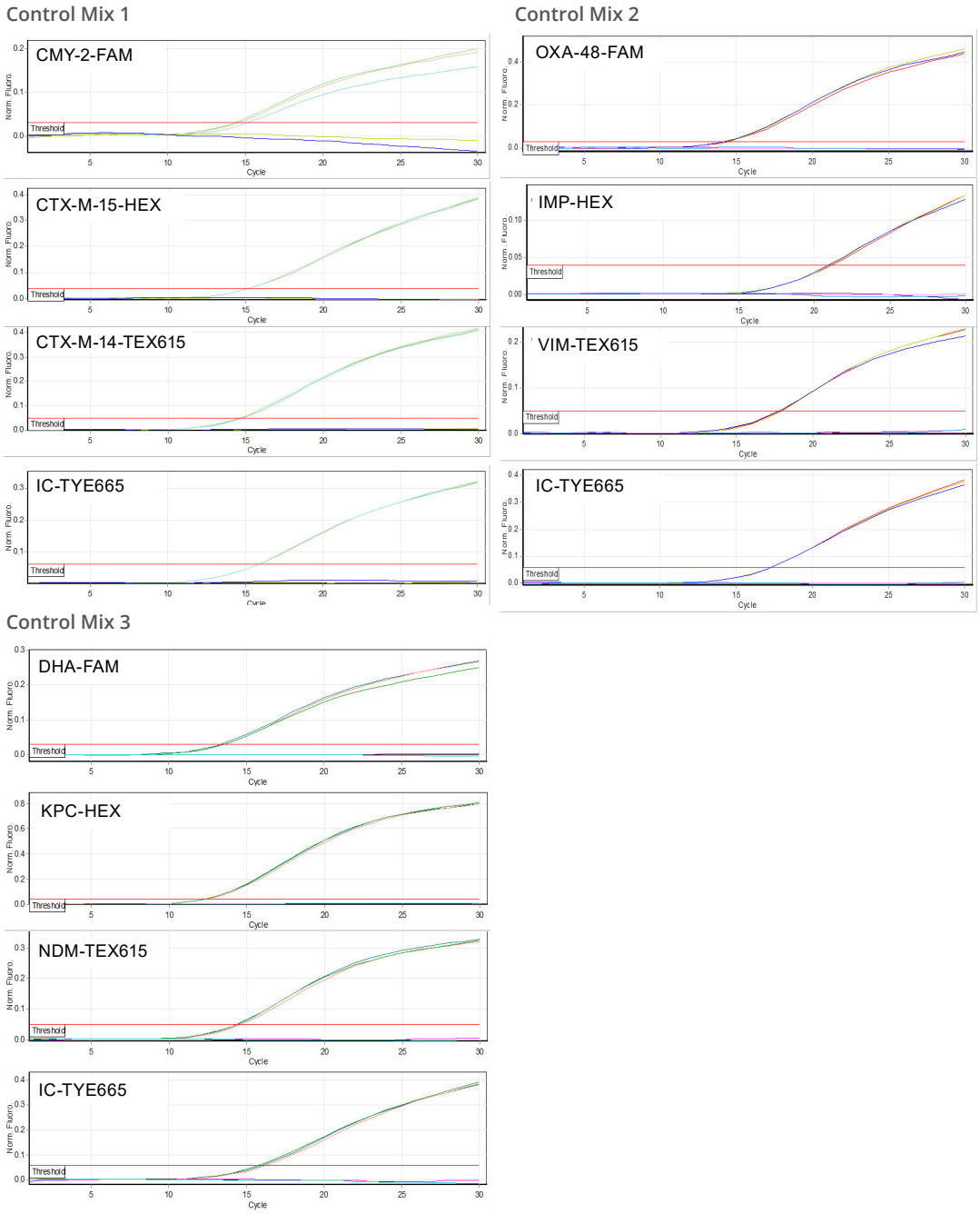


Figure 1: Multiplex real-time PCR amplification data of positive DNA Control Mixes for the Streck ARM-D Kit, ampC on the QIAGEN Rotor-Gene Q 5plex System.

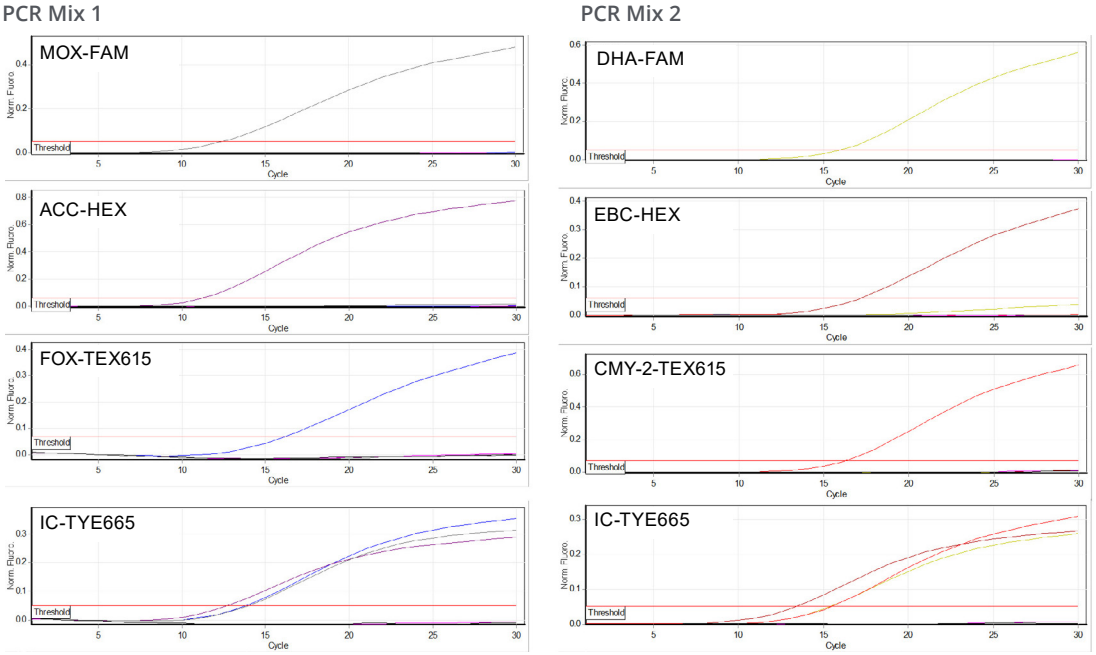


Figure 2: Amplification of plasmid-mediated ampC-positive clinical isolates using Streck ARM-D Kit, ampC. The data above shows amplification of six clinical isolates that are positive for each respective ampC target detected by the kit. The internal control (IC) was detected in each sample.

Cq Values – Controls

When setting threshold values for each target as specified in Table 1, Cq values obtained for positive controls during kit validation on the QIAGEN Rotor-Gene Q 5plex 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	Cq Value Range
Control Mix 1	MOX (FAM)	17 ± 3
	ACC (HEX)	18 ± 3
	FOX (TEX615)	21 ± 3
	IC (TYE665)	16 ± 3
Control Mix 2	DHA (FAM)	15 ± 3
	EBC (HEX)	18 ± 3
	CMY-2 (TEX615)	15 ± 3
	IC (TYE665)	15 ± 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.

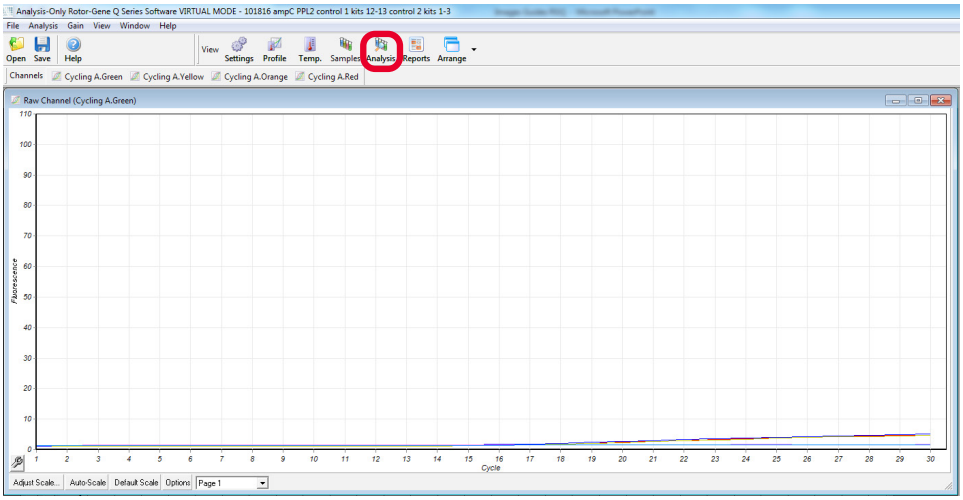
Data Analysis and Data Interpretation: Streck ARM-D Kit, β-Lactamase

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

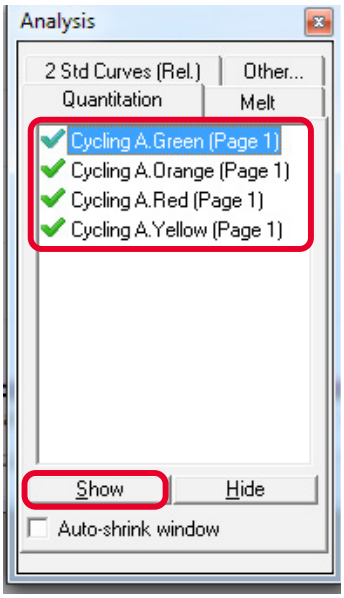
Prior to analyzing Cq values in the opened data file, threshold values and baseline settings for each fluorophore must be manually set following guidelines described in Table 3.

Click on the **Analysis** icon.



On the Analysis screen, select any of the optical channels.

Click **Show**.



***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.*

Enter the corresponding threshold value for the optical channel as it is specified in Table 3 (i.e., threshold value for FAM-Green Channel is 0.03).

Select the **Slope Correct** button (Note that **Dynamic Tube** button is selected by default).

Verify the baseline calculations look acceptable for the optical channel.

Repeat procedure for each optical channel.

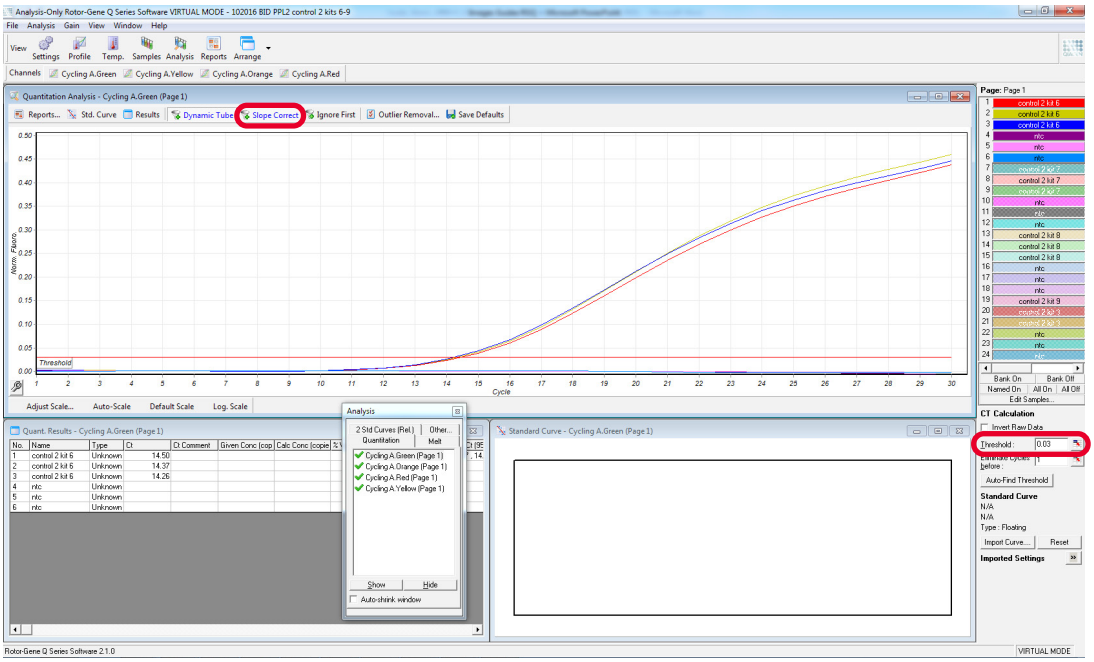
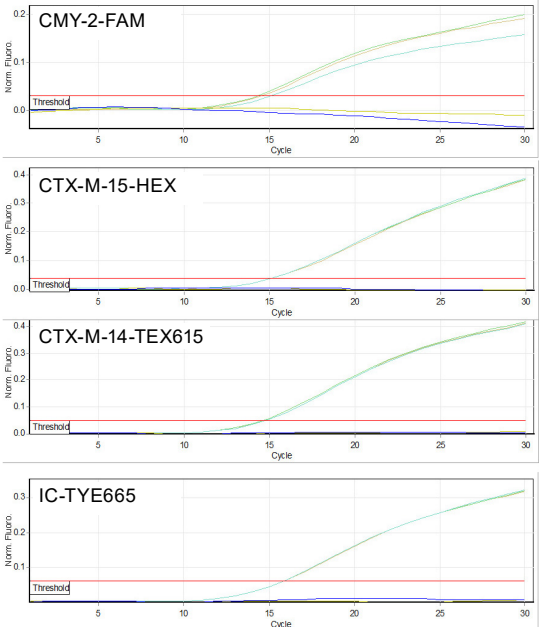


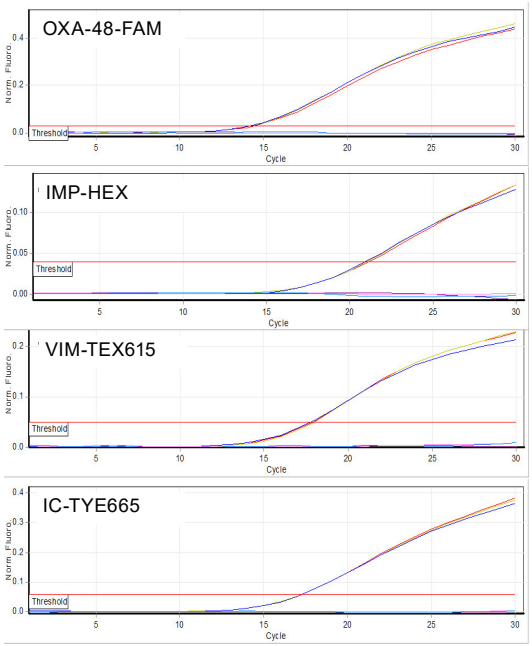
Table 3: Optical channels and threshold values determined during validation of the Streck ARM-D Kit, β-Lactamase on the QIAGEN Rotor-Gene Q 5plex System.

Master Mix	Target Gene Family	Fluorophore	Optical Channel	Threshold values
PCR Mix 1	CMY-2	FAM	Green	0.03
	CTX-M-15	HEX	Yellow	0.04
	CTX-M-14	TEX615	Orange	0.05
	IC	TYE665	Red	0.06
PCR Mix 2	OXA-48	FAM	Green	0.03
	IMP	HEX	Yellow	0.04
	VIM	TEX615	Orange	0.05
	IC	TYE665	Red	0.06
PCR Mix 3	DHA	FAM	Green	0.03
	KPC	HEX	Yellow	0.04
	NDM	TEX615	Orange	0.05
	IC	TYE665	Red	0.06

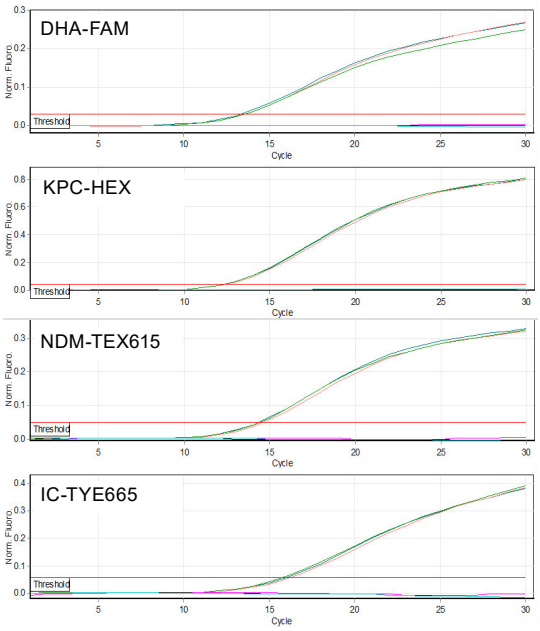
Control Mix 1



Control Mix 2



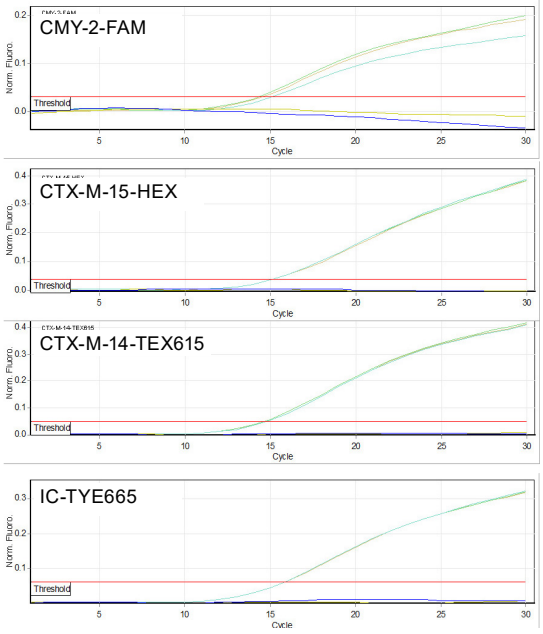
Control Mix 3



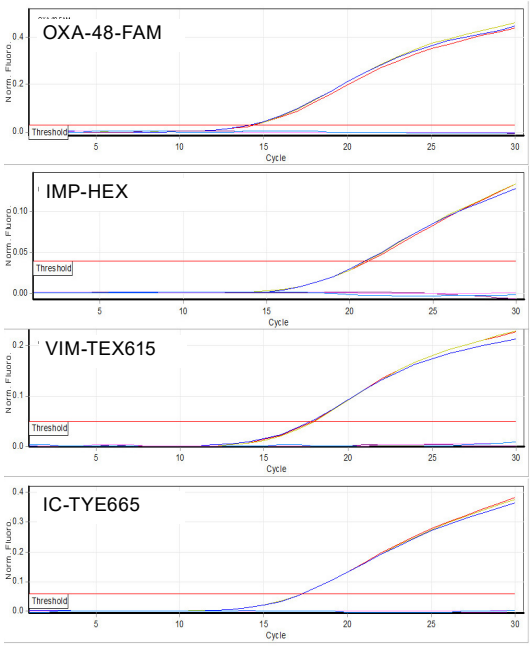
Amplification Curve Data

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, β-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 β-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

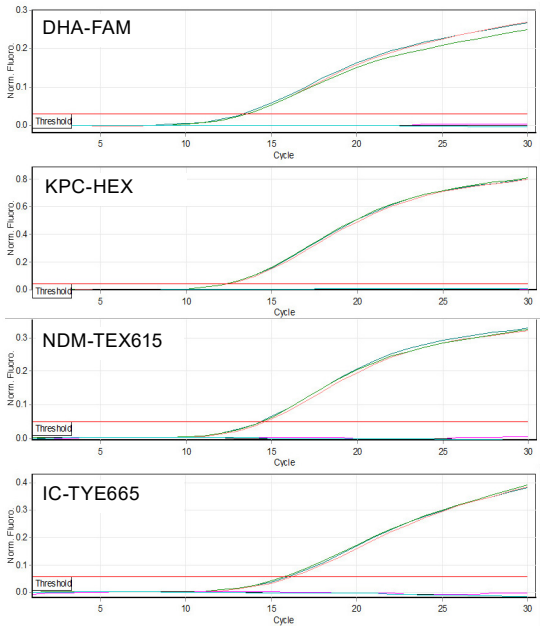
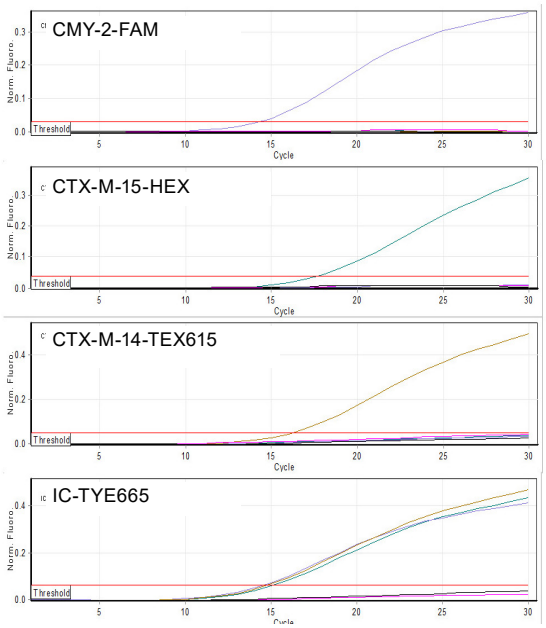
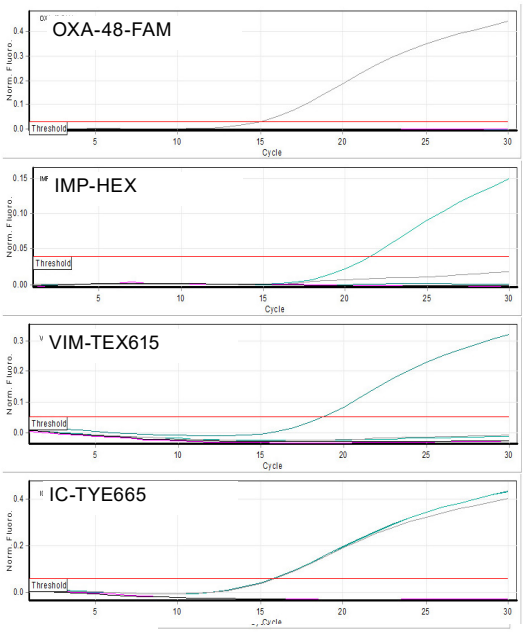


Figure 3: Multiplex real-time PCR amplification data of positive DNA Control Mixes (n=3) of Streck ARM-D Kit, β-Lactamase on the QIAGEN Rotor-Gene Q 5plex System.

PCR Mix 1



PCR Mix 2



PCR Mix 3

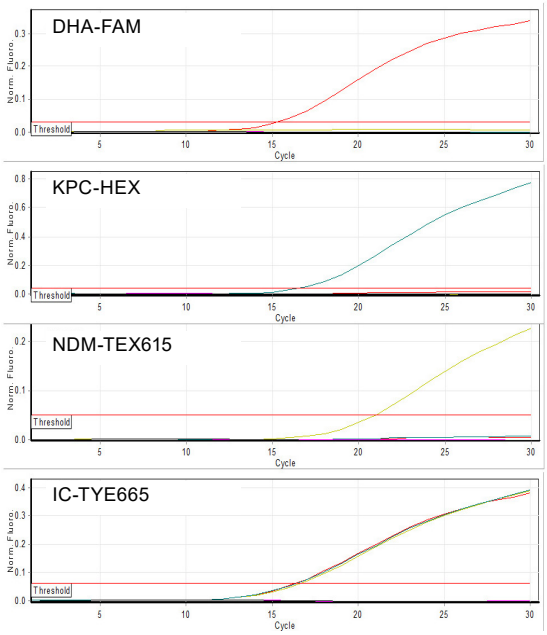


Figure 4: Amplification of β-lactamase-positive clinical isolates using Streck ARM-D Kit, β-Lactamase. Data shows the amplification of nine DNA samples that are positive for one of the respective β-Lactamase targets detected with the kit on the QIAGEN Rotor-Gene Q 5plex System. The internal control (IC) 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 QIAGEN Rotor-Gene Q 5plex 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.

Table 4: Cq values for positive control targets determined during validation of the Streck ARM-D Kit, β-Lactamase on the QIAGEN Rotor-Gene Q 5plex System.

Control Mix	Target Gene Family (Fluorophore)	Cq Value Range
Control Mix 1	CMY-2 (FAM)	14 ± 3
	CTX-M-15 (HEX)	15 ± 3
	CTX-M-14 (TEX615)	15 ± 3
	IC (TYE665)	16 ± 3
Control Mix 2	OXA-48 (FAM)	14 ± 3
	IMP (HEX)	17 ± 3
	VIM (TEX615)	16 ± 3
	IC (TYE665)	16 ± 3
Control Mix 3	DHA (FAM)	14 ± 3
	KPC (HEX)	12 ± 3
	NDM (TEX615)	14 ± 3
	IC (TYE665)	15 ± 3

Cq Values - Unknown Samples

To classify unknown samples as positive or negative for β-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 β-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/µL purified DNA samples.

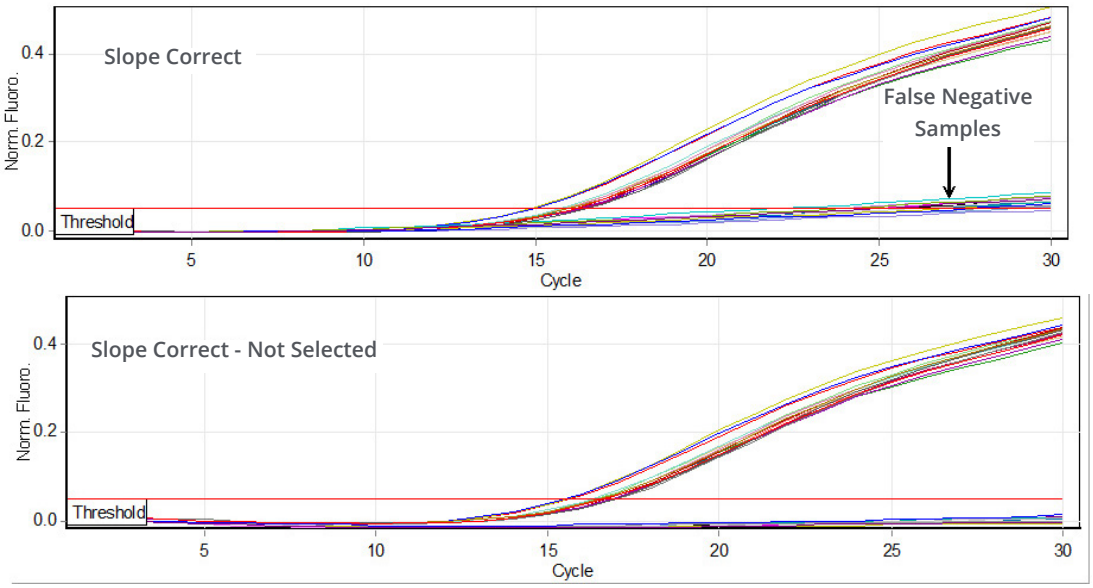
Positive Sample: Overall, unknown samples (using 10-200 ng/µL DNA in a PCR) may be interpreted as positive if the Cq value is ≤ 26 cycles.

Negative Sample: If no Cq value is detected in the Green, Yellow, and Orange 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 Red channel (Cq = 10 - 20). If IC (Red channel) is amplified and no amplification is detected in Green, Yellow, and Orange channels, the unknown sample may be interpreted as negative for the respective resistance mechanisms appropriate for each probe provided within the kit.

Invalid/Flag for Further Investigation: If amplification of an unknown sample in the Green, Yellow, Orange, and Red channels is detected after 26 cycles or if IC is not detected, then the sample requires further investigation. The sample may be re-extracted, the PCR run repeated, or the amplified product could be sequenced for verification.

Troubleshooting

Incorrect baseline settings: For some samples, baseline settings that are erroneously assigned may cause false positive or false negative values. In the example shown below, baseline calculations after selecting Slope Correct button, as described in the data analysis section, identify several negative samples as positive samples although there is no PCR curve (top image). This can be corrected by de-selecting Slope Correct button (bottom image). It is highly recommended that amplification data for each optical channel is visually inspected for proper data interpretation.



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**.