

## Streck ARM-D<sup>®</sup> Kits

### Data Acquisition and Analysis Guide

Platform: Bio-Rad CFX96 Touch Real-Time PCR Detection System

#### Quick Links

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

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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 the analysis of your data following a PCR run. Refer to the instrument manual for a detailed description on the instrument's operation and data analysis.

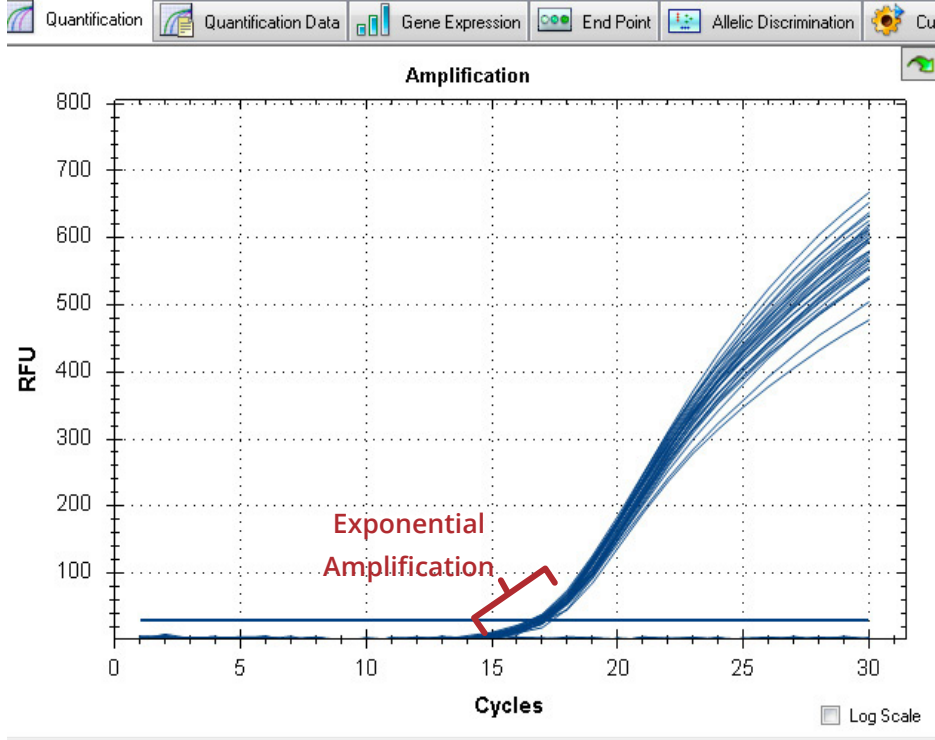
#### General Recommendations

**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.

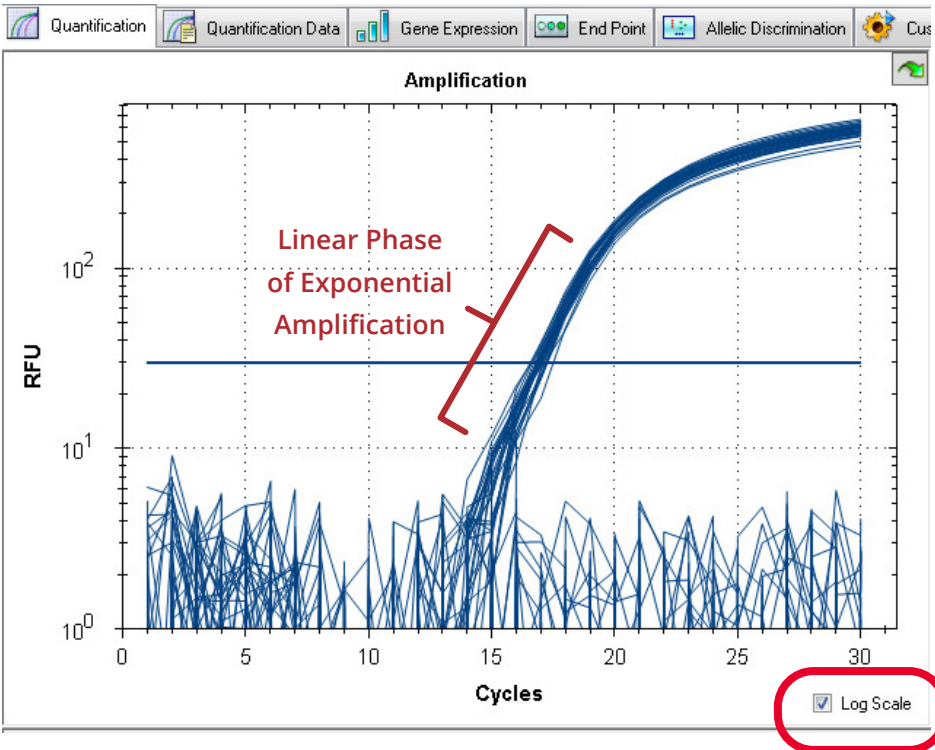
**Threshold Settings:** Although automatic analysis is often appropriate, manually setting threshold values is more convenient for consistent comparisons between runs. Recommended settings for fluorophore-specific thresholds (Tables 1 and 3) are provided in this document, based on data acquired during 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 examples).

# Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System

## Linear Scale View



## Log Scale View



## Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System

**Baseline Settings:** Similar to the threshold settings, automatic baseline settings often give acceptable results, but manually defining the baseline begin and end cycles may help avoid software errors that could affect data interpretation. Automatic baseline cycle settings were used during kit validation when using the same DNA concentrations as described in the IFU (10-200 ng/μL of bacterial DNA). However, it may be necessary to adjust the baseline settings following data evaluation. To adjust the baseline cycles manually for each fluorophore or target, note which reaction is the first with fluorescence that exceeds the visible baseline level; then determine at which cycle 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 controls and unknown samples should demonstrate amplification between cycles 10 and 26. Cq values determined 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 obtained results.

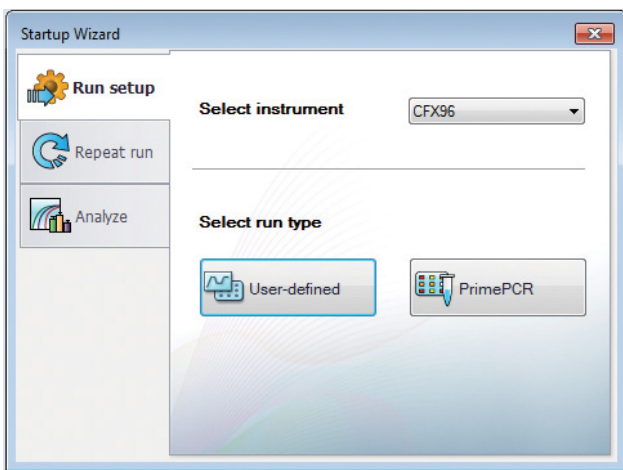
Specific data analysis instructions are provided for the Streck ARM-D Kit, *ampC* and Streck ARM-D Kit, β-Lactamase.

### Instrument Set-up

Open Bio-Rad CFX Manager Software.

On the Startup Wizard screen, select **Run setup**.

Select **User-defined** run type.

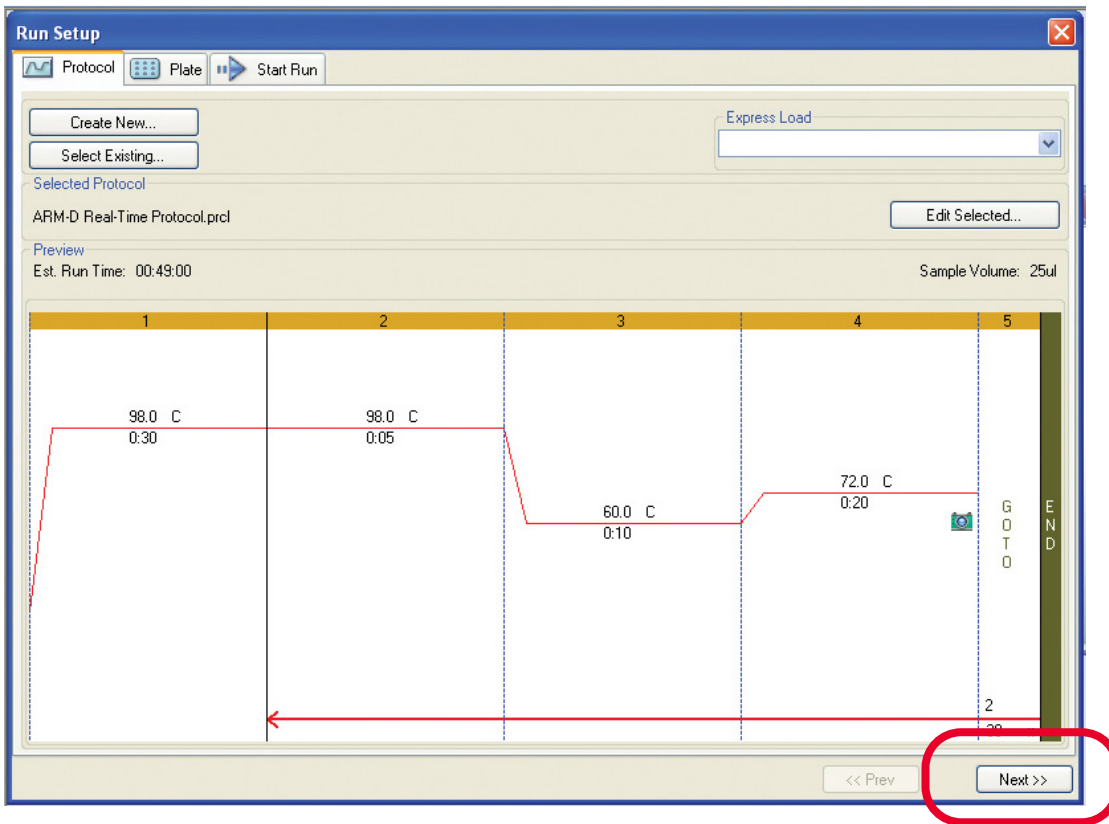


Create New Protocol. Set-up ARM-D Kit protocol using the times and temperatures listed below. Note the PCR cycling protocol is the same for both the *ampC* and β-Lactamase Streck ARM-D Kits.

ARM-D Kit Real-Time PCR Cycling Protocol		
Hot-start	Step 1	98 °C for 30 sec
30 cycles of	Step 2	98 °C for 5 sec
	Step 3	60 °C for 10 sec
	Step 4	72 °C for 20 sec (Detection Step)

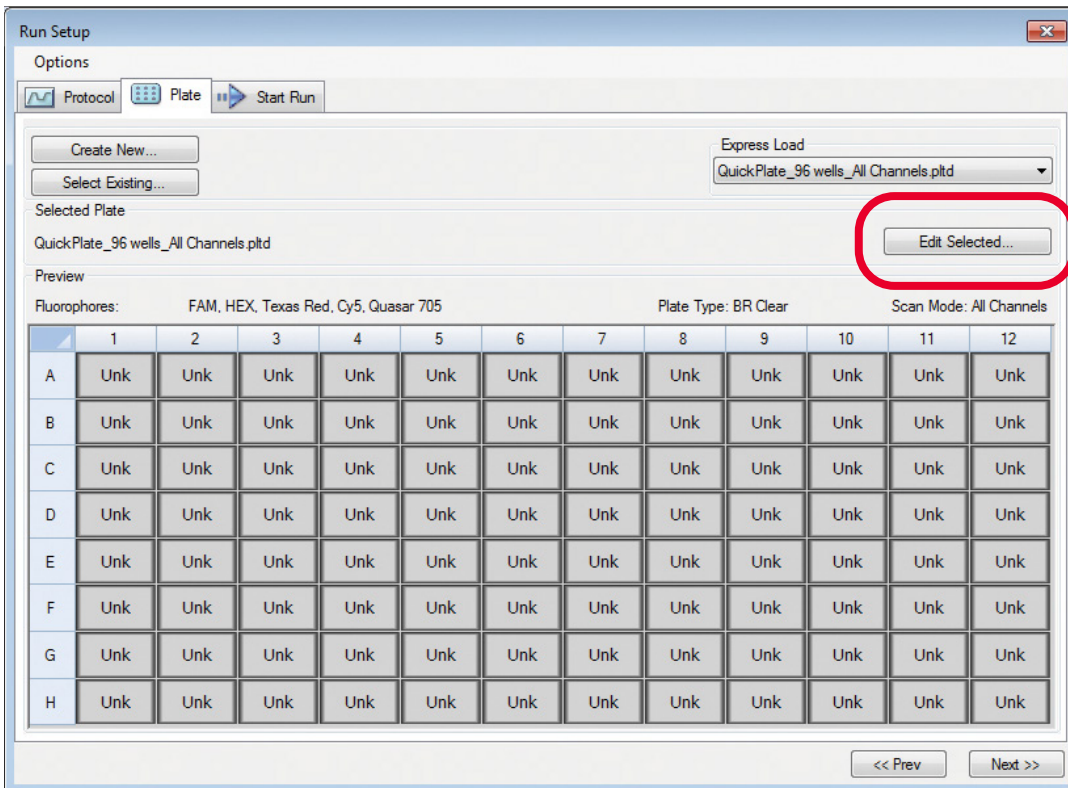
- Sample volume: 25μL.
- Ensure the "Plate Read (camera icon)" is selected to read after the extension/detection step. In the next image, it is located at the end of Step 4.

# Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System



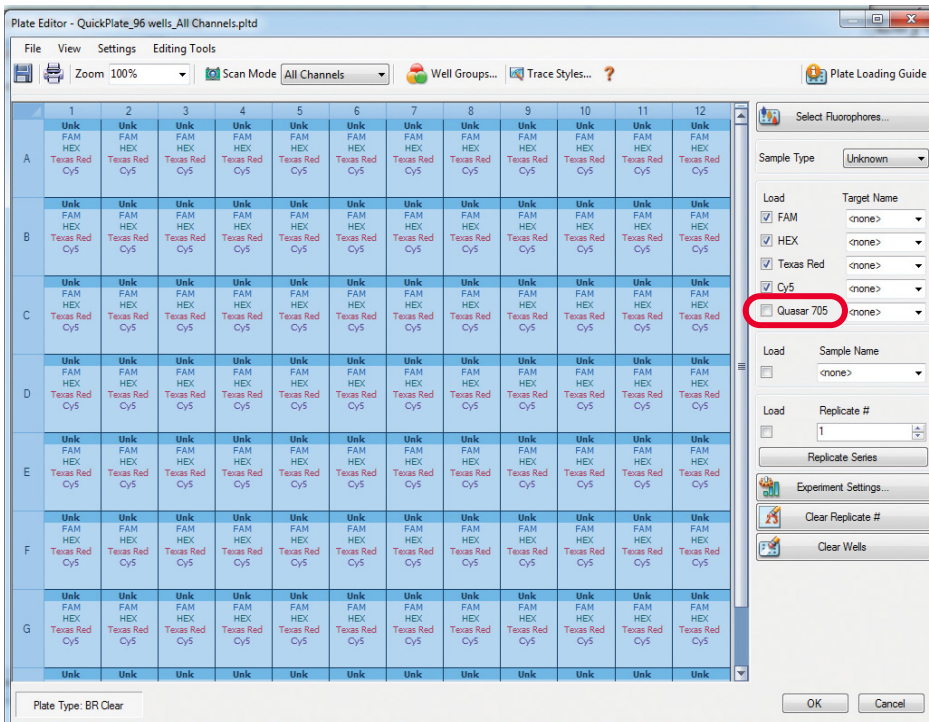
Click Next.

On the Plate tab, click the Edit Selected tab to change the default Quick Plate\_96-wells\_All Channels plate layout.



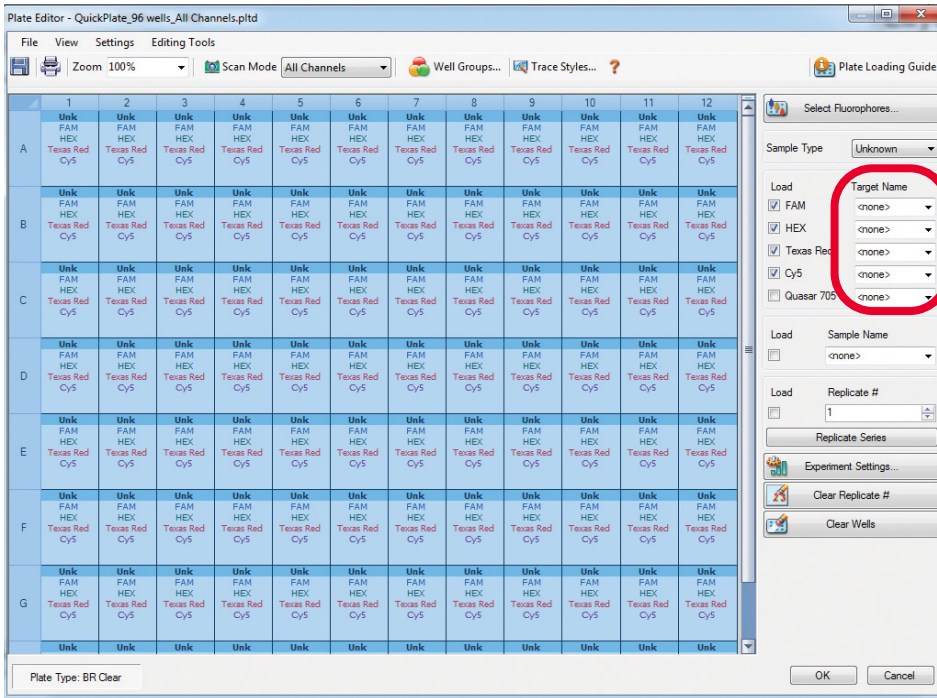
# Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System

In the Plate Editor Window, select all the wells in the layout and de-select Fluorophore Quasar 705 box (Channel 5). This removes the unnecessary optical channel and leaves only the optical channels specific to each fluorophore used in the Streck ARM-D Kits.



\*Optional: Edit Sample Types on the layout and/or identify targets for each PCR Mix by their respective fluorophore/optical channel combinations as described below in Table 1, *ampC* Kit, and Table 3,  $\beta$ -Lactamase Kit, (Data Analysis section).

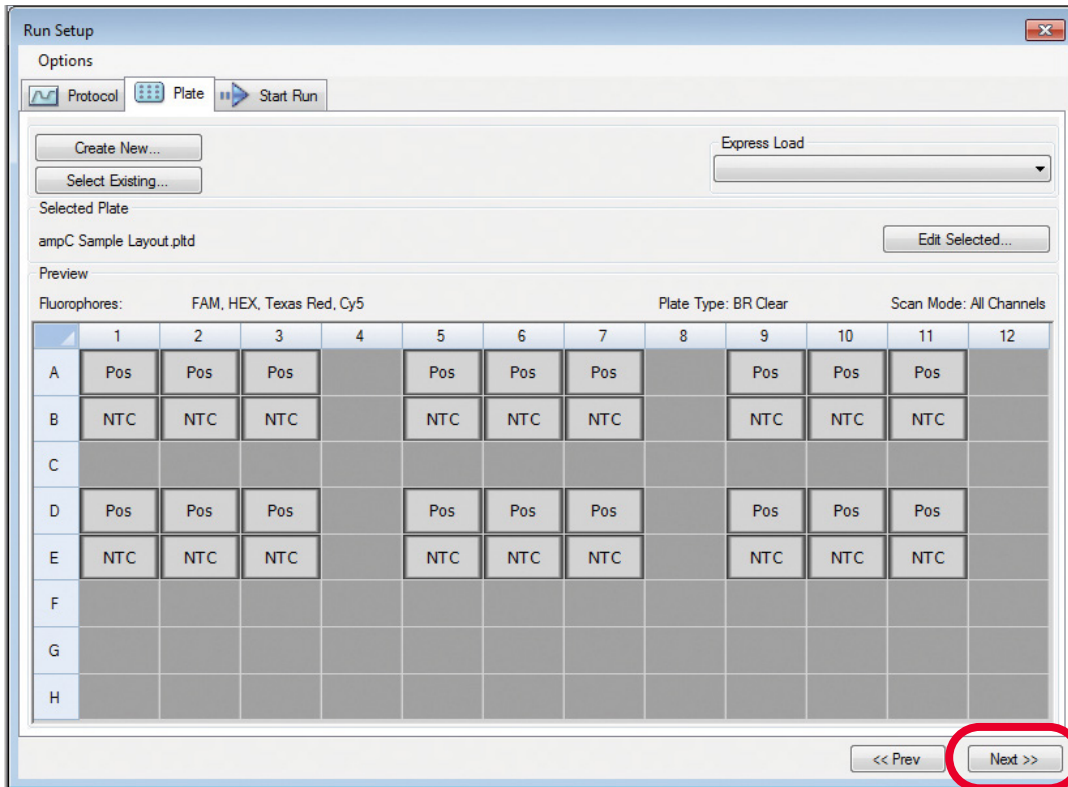
To assign a name for specific targets, type the target name into the box next to the appropriate optical channel, then click the Load box next to each channel. This permits thresholds and baselines to be set and analyzed specific to each target detected by each PCR Mix.



# Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System

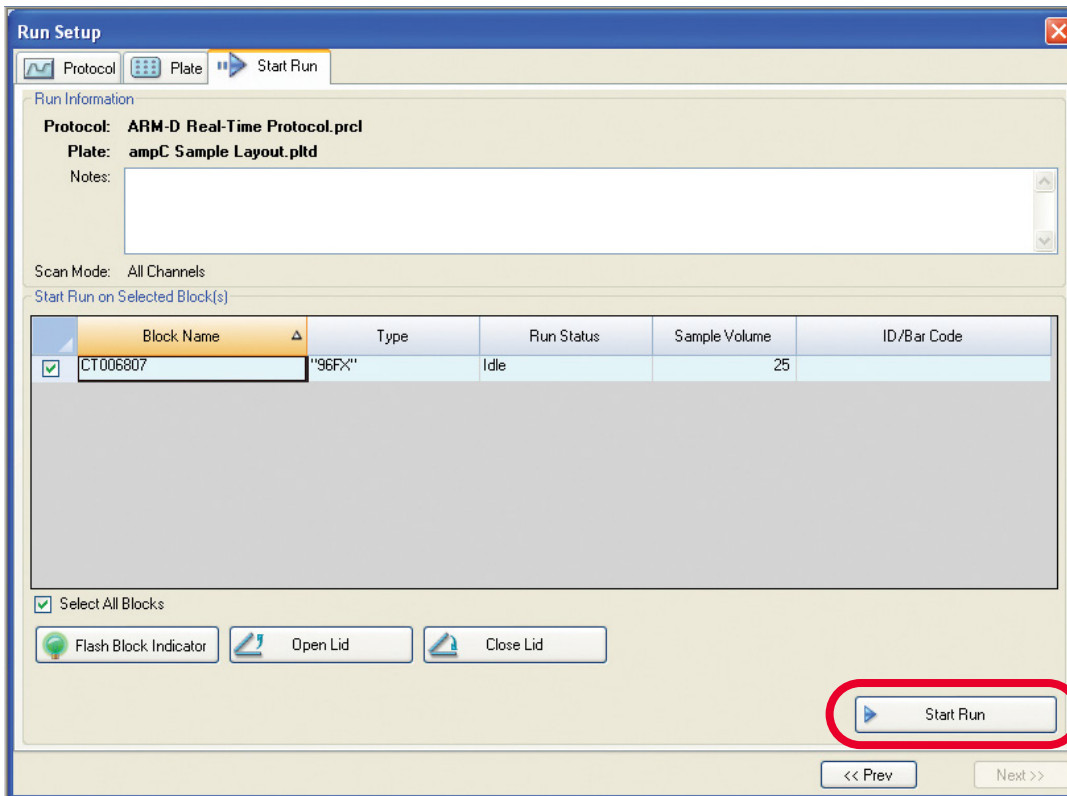
Click OK to save changes.

Click Next.



## Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System

Click Start Run. The run should be complete in approximately 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. Quantification cycle (C<sub>q</sub>) values for the positive controls should fall within the range recommended in the Instructions For Use. If C<sub>q</sub> values fall outside the 10 to 26 cycle range, data should be interpreted carefully prior to confirming a positive or negative result. Expected positive control C<sub>q</sub> values are provided in Table 2. These values were determined during validation of the kits using the recommended baseline/threshold settings listed in Table 1 and are meant to be used as a point of reference. These values may change on a case-by-case basis.

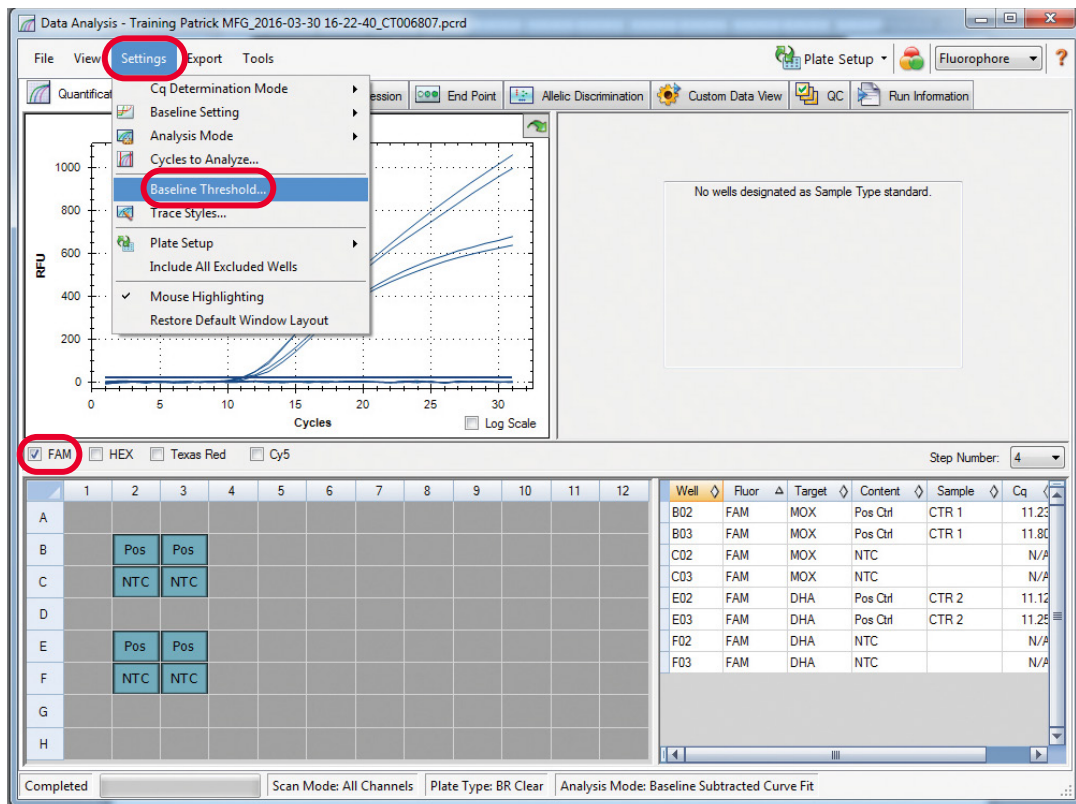
#### Threshold values

After opening the corresponding data file in the Bio-Rad CFX Manager and prior to analyzing C<sub>q</sub> values, threshold values for each fluorophore should be manually set as described in Table 1. Note: If target names specific to each PCR Mix were entered in the Plate Setup menu, then the optimal threshold values for each target may also be set.

## Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System

Select one fluorophore channel (i.e., FAM, HEX, Texas Red, or Cy5) at a time on the Data Analysis screen on the Quantification tab.

Click Settings and go to Baseline Threshold.



The screenshot shows the 'Data Analysis' software interface. The 'Settings' menu is open, and 'Baseline Threshold...' is selected. The 'FAM' channel is selected in the bottom left. The main window shows a graph of RFU vs Cycles and a table of results.

Well	Fluor	Target	Content	Sample	Cq
B02	FAM	MOX	Pos Ctrl	CTR 1	11.23
B03	FAM	MOX	Pos Ctrl	CTR 1	11.80
C02	FAM	MOX	NTC		N/A
C03	FAM	MOX	NTC		N/A
E02	FAM	DHA	Pos Ctrl	CTR 2	11.12
E03	FAM	DHA	Pos Ctrl	CTR 2	11.25
F02	FAM	DHA	NTC		N/A
F03	FAM	DHA	NTC		N/A

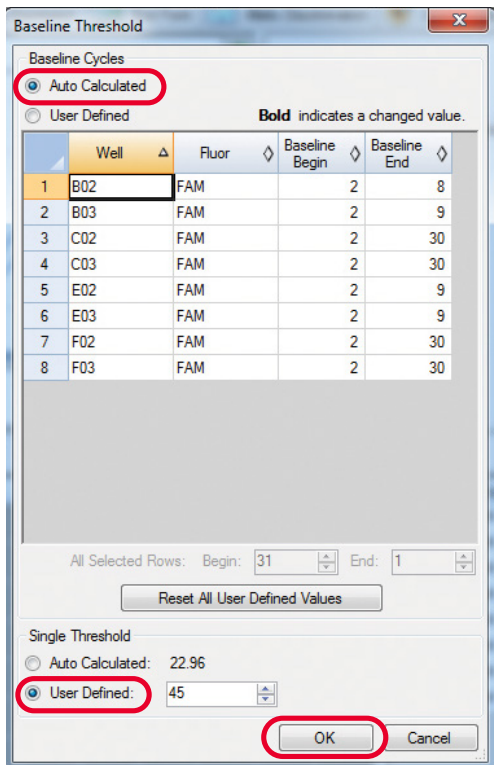
On the Baseline Threshold screen, leave the Baseline Cycles selected for **Auto Calculated**.

For Single Threshold select **User Defined**.

Enter the corresponding threshold value for the channel specified in Table 1 (i.e., FAM is 45).

Click **OK**.

## Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System



Repeat procedure for each fluorophore using the values listed in Table 1. Threshold values can be changed if a more optimal value is determined upon review of the data.

**Table 1:** Optical channels and threshold values for data analysis of Streck ARM-D Kit, *ampC* on the Bio-Rad CFX96.

Master Mix	Target Gene Family	Fluorophore	Optical Channel	Threshold Value
PCR Mix 1	MOX	FAM	FAM	45
	ACC	HEX	HEX	30
	FOX	TEX615	Texas Red	60
	IC	TYE665	Cy5	30
PCR Mix 2	DHA	FAM	FAM	45
	EBC	HEX	HEX	30
	CMY-2	TEX615	Texas Red	60
	IC	TYE665	Cy5	30

### Amplification Curve Data

After setting threshold values, PCR amplification curves for every sample should be visually inspected to confirm amplification of the sample and that the baseline and threshold settings are optimal 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 for specific guidelines on interpreting unknown sample data.

# Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System

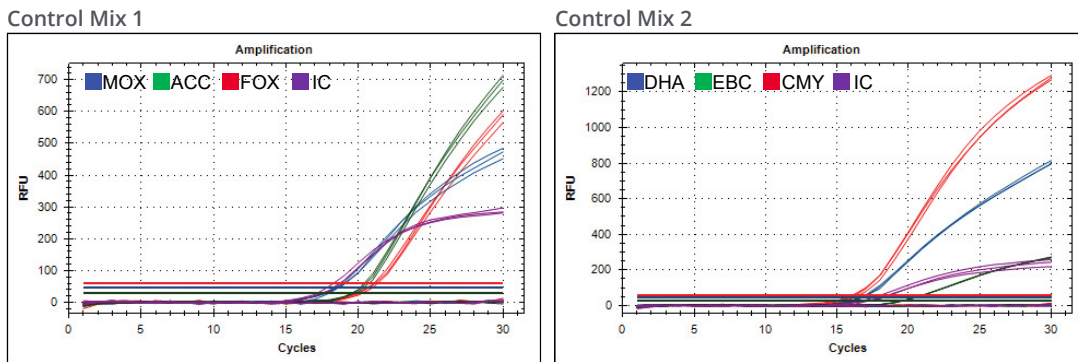


Figure 1: Multiplex real-time PCR amplification data of positive DNA Control Mixes (n=3) of Streck ARM-D Kit, *ampC* on the Bio-Rad CFX96 Instrument.

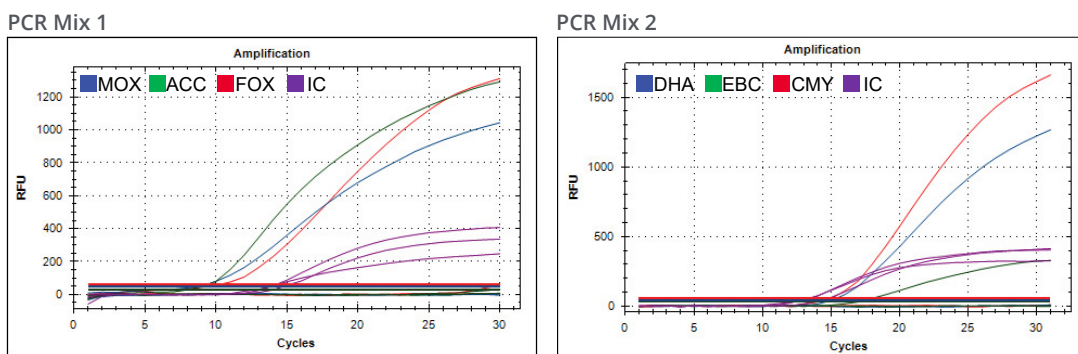


Figure 2: Amplification of plasmid-mediated *ampC*-positive clinical isolates using Streck ARM-D Kit, *ampC*. The data above shows amplification of six clinical isolates that are positive for each respective *ampC* target detected by the kit. The IC (purple lines) was detected in each sample.

## Cq Values – Controls

When setting threshold values based on each fluorophore as specified in Table 1, Cq values obtained for positive controls during kit validation on the Bio-Rad CFX96 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 target identification with the Streck ARM-D Kit, *ampC*.

Control Mix	Target Gene Family (Fluorophore)	Cq Value Range
Mix 1	MOX (FAM)	18 ± 3
	ACC (HEX)	20 ± 3
	FOX (TEX615)	21 ± 3
	IC (TYE665)	17 ± 3
Mix 2	DHA (FAM)	17 ± 3
	EBC (HEX)	20 ± 3
	CMY-2 (TEX615)	17 ± 3
	IC (TYE665)	18 ± 3

# Streack ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System

## 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: Streack ARM-D Kit, β-Lactamase

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

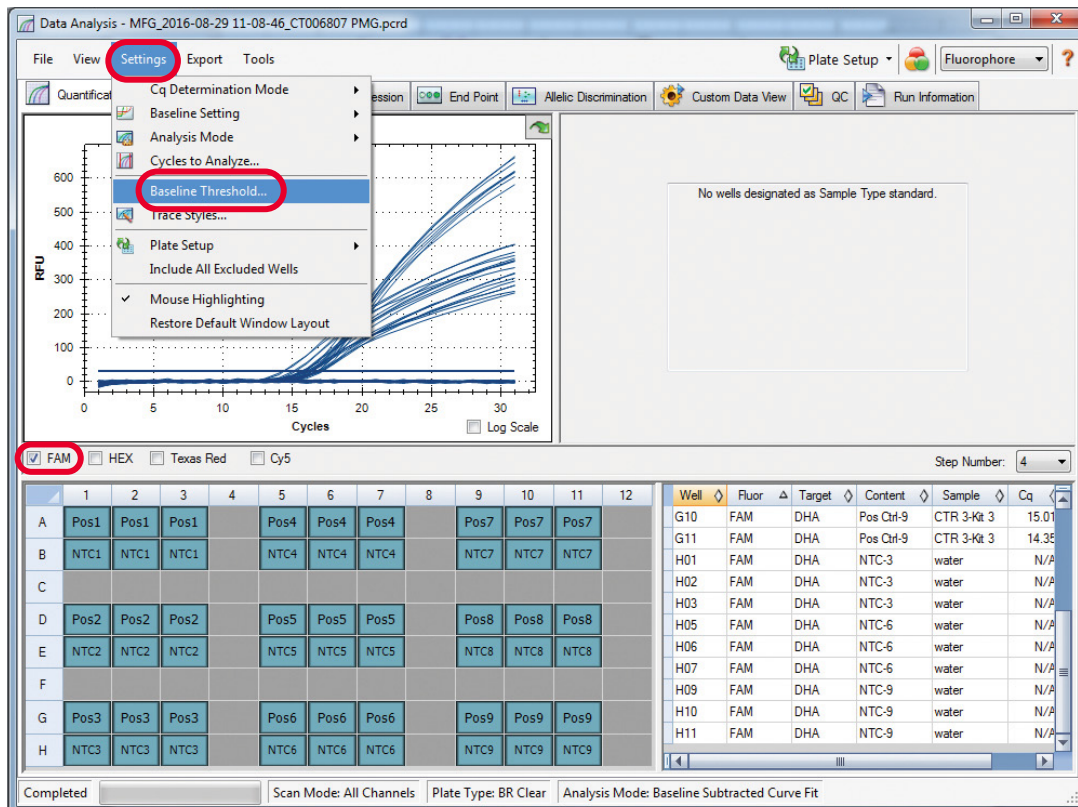
### Threshold values

After opening the corresponding data file in the Bio-Rad CFX Manager and prior to analyzing Cq values, threshold values for each fluorophore should be manually set as described in Table 3.

**Note:** If target names specific to each PCR Mix were entered in the Plate Setup menu, then the optimal threshold values for each target may also be set.

Select one fluorophore channel (i.e., FAM, HEX, Texas Red, or Cy5) at a time on the Data Analysis window on the Quantification tab.

Click **Settings** and go to **Baseline Threshold**.



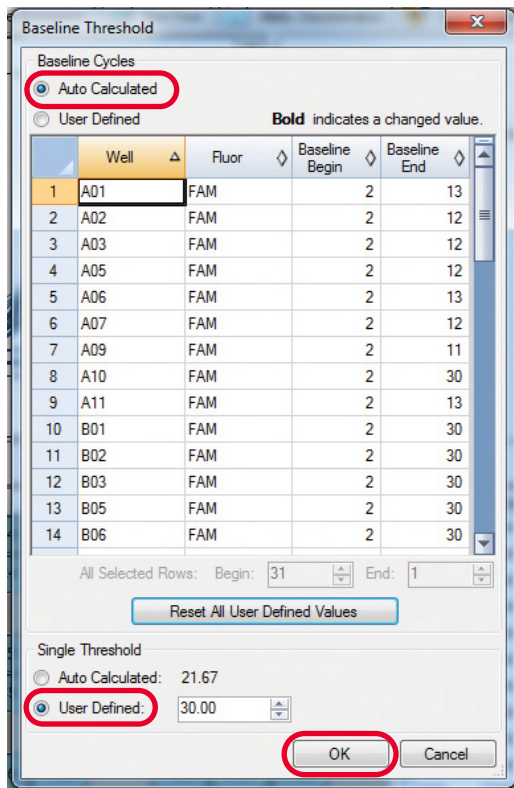
On the Baseline Threshold screen, leave the Baseline Cycles selected for **Auto Calculated**.

For Single Threshold select **User Defined**.

Enter the corresponding threshold value for the channel specified in Table 1 (i.e., FAM is 30).

Click **OK**.

## Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System



Repeat procedure for each fluorophore using the values described in Table 3.

**Table 3:** Optical channels and threshold values for data analysis of Streck ARM-D Kit,  $\beta$ -Lactamase on the Bio-Rad CFX96.

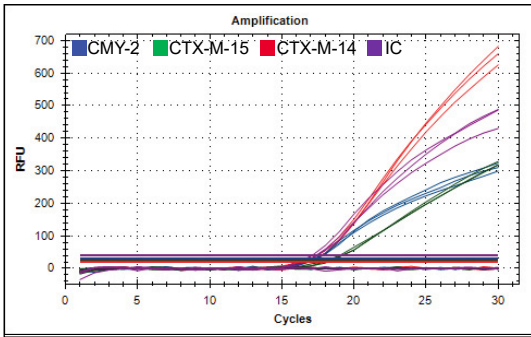
Master Mix	Target Gene Family	Fluorophore	Optical Channel	Threshold Values
PCR Mix 1	CMY-2	FAM	FAM	30
	CTX-M-15	HEX	HEX	40
	CTX-M-14	TEX615	Texas Red	35
	IC	TYE665	Cy5	40
PCR Mix 2	OXA-48	FAM	FAM	30
	IMP	HEX	HEX	40
	VIM	TEX615	Texas Red	35
	IC	TYE665	Cy5	40
PCR Mix 3	DHA	FAM	FAM	30
	KPC	HEX	HEX	40
	NDM	TEX615	Texas Red	35
	IC	TYE665	Cy5	40

### Amplification Curve Data

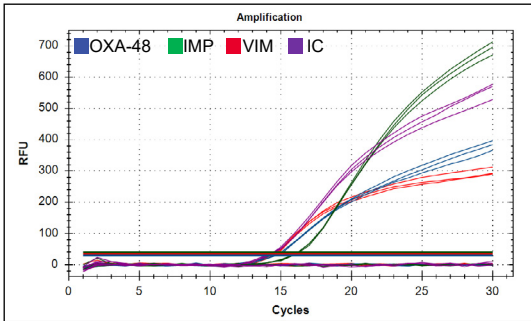
After setting threshold values, PCR amplification curves for every sample should be visually inspected to confirm amplification of the sample and that the baseline and threshold settings are optimal for analysis of the data. Characteristic amplification data for positive control targets of Streck ARM-D 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 for specific guidelines on interpreting unknown sample data.

# Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System

Control Mix 1



Control Mix 2



Control Mix 3

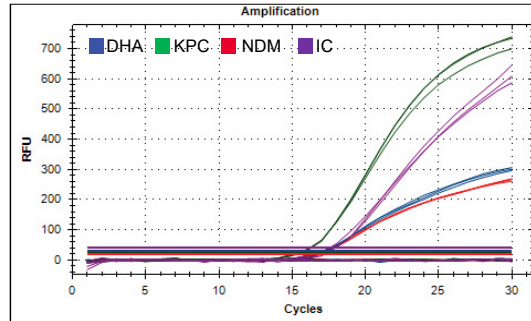
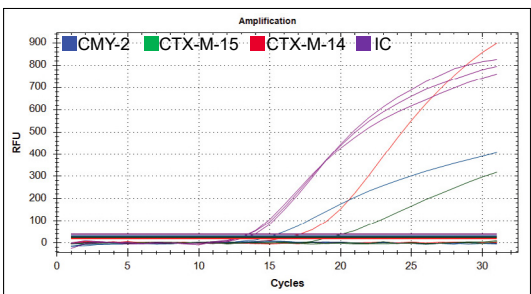
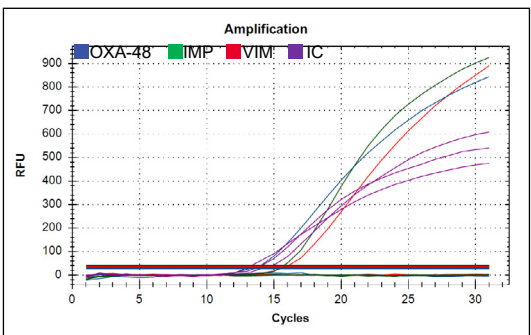


Figure 3: Multiplex real-time PCR amplification data of positive DNA Control Mixes (n=3) of Streck ARM-D Kit,  $\beta$ -Lactamase on the Bio-Rad CFX96 Instrument.

PCR Mix 1



PCR Mix 2



PCR Mix 3

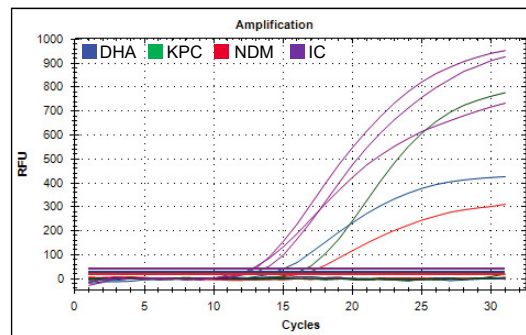


Figure 4: Amplification of  $\beta$ -lactamase-positive clinical isolates using Streck ARM-D Kit,  $\beta$ -Lactamase. Data obtained on the Bio-Rad CFX96 instrument shows the amplification of nine clinical isolates that are positive to a  $\beta$ -lactamase target. The IC was identified on each isolate accordingly.

## Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System

### Cq Values – Controls

After setting threshold values based on each fluorophore as specified in Table 3, Cq values obtained for positive controls during kit validation on the Bio-Rad CFX96 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 target identification with the Streck ARM-D Kit, β-Lactamase.

Control Mix	Target Gene Family	Cq Value Range
Control Mix 1	CMY-2 (FAM)	17 ± 3
	CTX-M-15 (HEX)	19 ± 3
	CTX-M-14 (TEX615)	17 ± 3
	IC (TYE665)	17 ± 3
Control Mix 2	OXA-48 (FAM)	15 ± 3
	IMP (HEX)	16 ± 3
	VIM (TEX615)	15 ± 3
	IC (TYE665)	16 ± 3
Control Mix 3	DHA (FAM)	17 ± 3
	KPC (HEX)	16 ± 3
	NDM (TEX615)	17 ± 3
	IC (TYE665)	18 ± 3

### Cq Values – Unknown samples

To classify unknown samples as positive or negative for β-Lactamase targets, refer to the **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 10-200ng/μL purified DNA samples.

### Interpretation

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

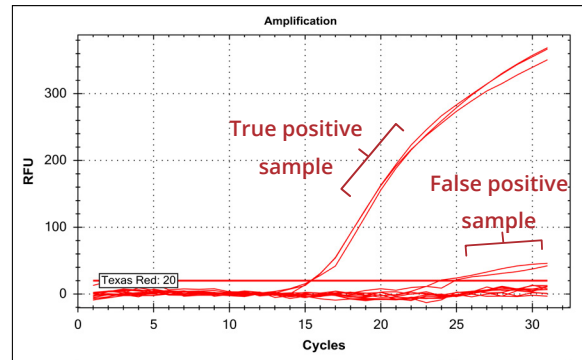
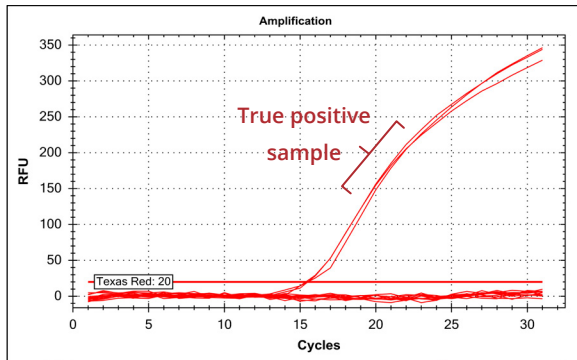
**Negative Sample:** If no Cq value is detected in the FAM, HEX, and Texas Red channels for unknown samples, confirm sample was added to the reactions by verifying positive amplification of the internal control (IC) in the Cy5 channel (Cq = 10-20). If IC (Cy5) is amplified and no amplification is detected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

## Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System

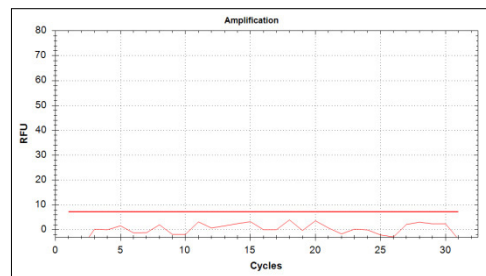
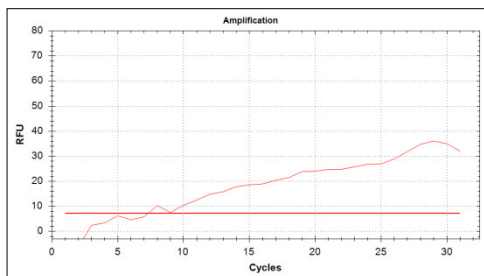
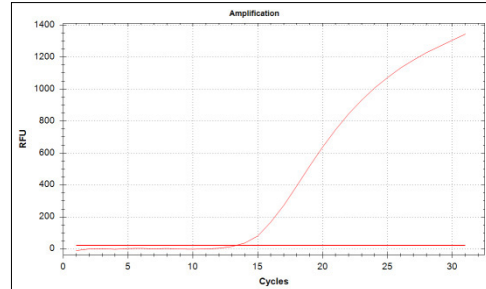
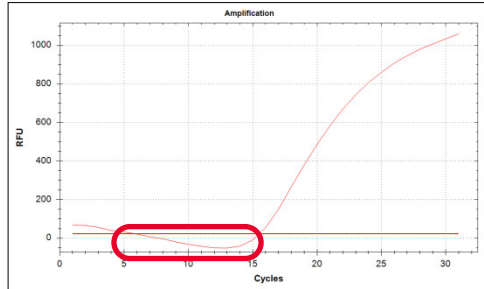
**Invalid/Flag for Further Investigation:** If amplification of an unknown sample in the FAM, HEX, Texas Red, and Cy5 channels is detected after 26 cycles or if the IC is not detected, then sample requires further investigation. The sample may be re-extracted, the PCR run repeated, or the amplified product could be sequenced for verification.

### Troubleshooting

- 1. False positive samples in the Texas Red channel:** If Quasar 705 channel (optical channel 5 Bio-Rad CFX96) is not de-selected during instrument set up, it may cause higher background readings on the Texas Red channel. This may cause false positive samples in targets conjugated to Texas Red as the background is not properly calculated when Quasar 705 channel is selected as shown in the example below. This can be corrected after data is collected (i.e., Quasar 705 channel can be de-selected during data analysis).

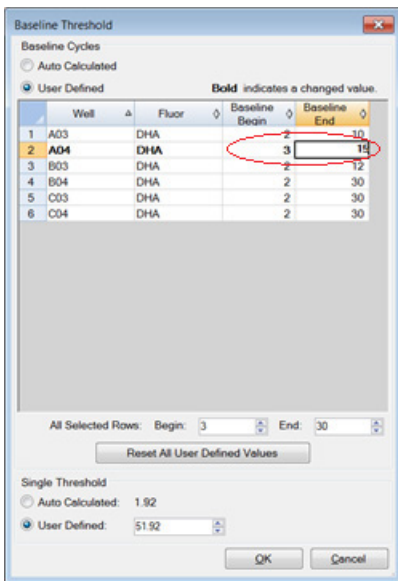


- 2. Incorrect baseline settings:** For some samples, automatic baseline settings that are erroneously assigned may cause false positive or false negative values. Usually these are noticeable in the amplification plots, as in the examples below:



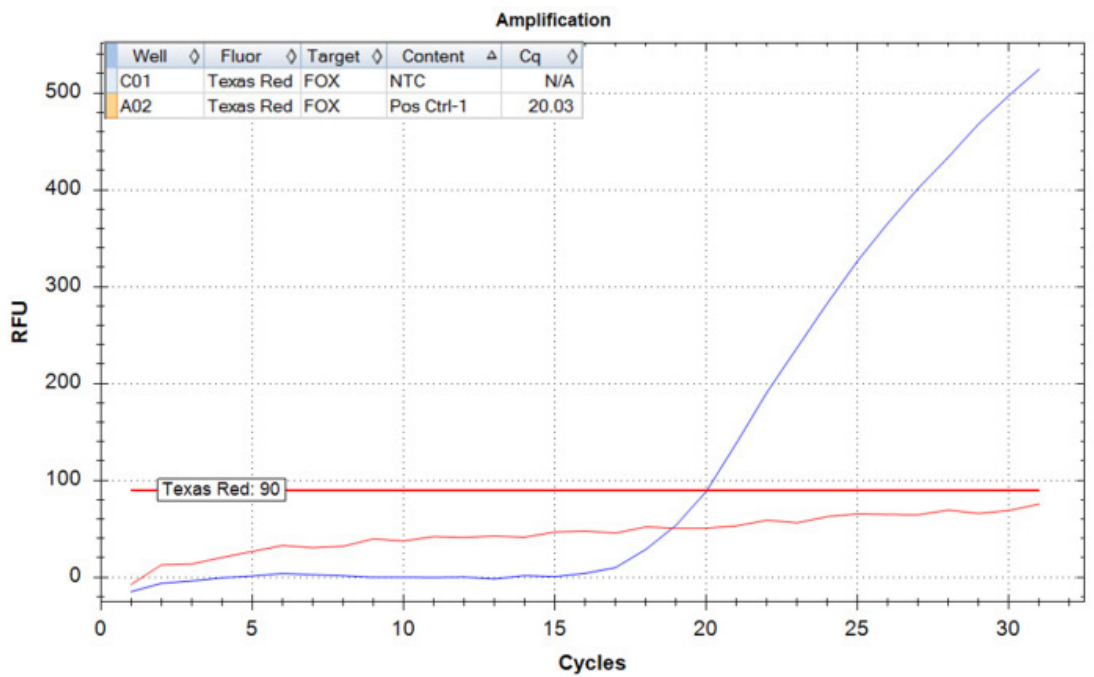
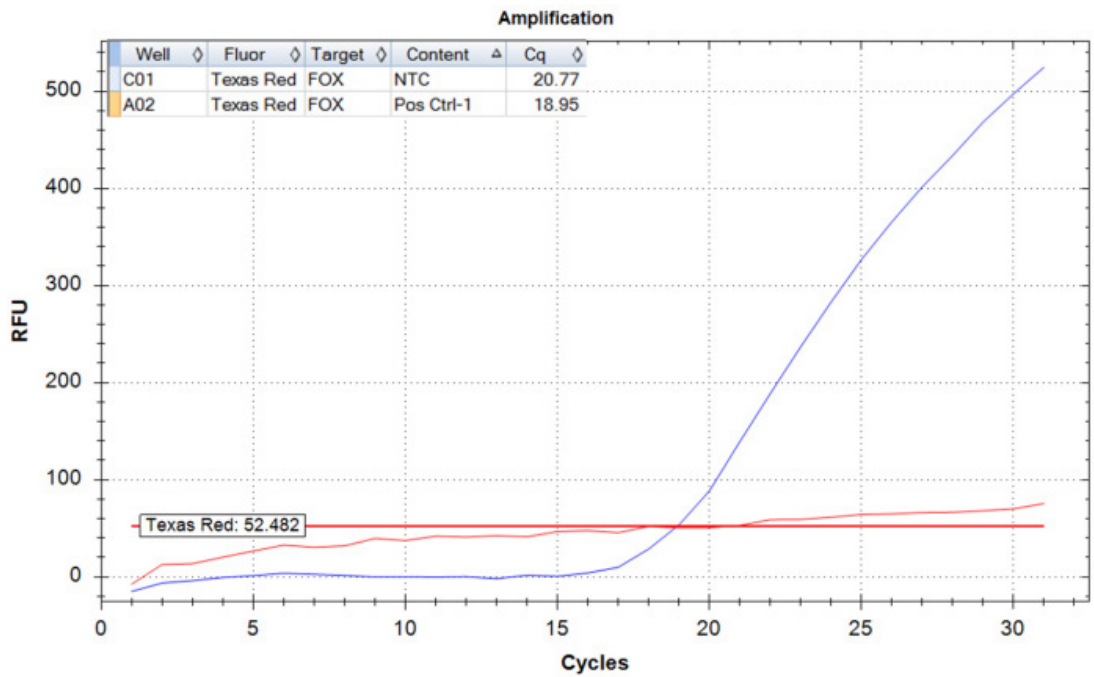
If upon visual inspection it is apparent that one or more amplification curves aren't properly processed by the software, the baseline begin and end cycles should be manually adjusted for those samples to ensure proper C<sub>q</sub> calculations. (Select the fluorophore/target for the plot being corrected > **Settings** > **Baseline Threshold** > select the appropriate well and type the proper **Baseline Begin** and **Baseline End** cycles > **OK**). Verify that the amplification plot appearance was corrected.

## Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System



**False amplification:** On occasion, some apparent increase in fluorescence that is not caused by target amplification may exceed threshold levels and 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, two samples (blue and red lines) result in low Cq values (18.95 and 20.77 respectively), indicating the presence of the specific target. However, on examination of the data, if it becomes apparent that while the blue amplification plot corresponds to a typical PCR amplification and can be interpreted as a positive sample, the red plot contains artifacts and should be considered a negative sample. In this case, a slightly higher threshold (set at 90 RFU) would have resulted in the correct interpretation.

# Streck ARM-D® Kits: Bio-Rad CFX96 Touch Real-Time PCR Detection System



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).