

Streck ARM-D[®] Kits

Data Acquisition and Analysis Guide

**Real-Time PCR Platform:
Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR System**

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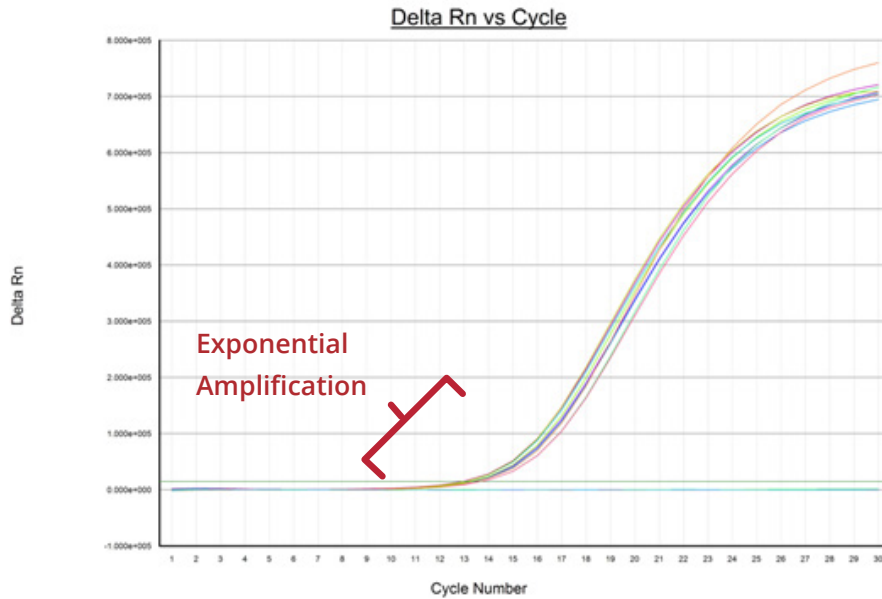
This guide is intended to be used as a Streck ARM-D Kit-specific supplement for the Instructions For Use (IFU) document. The kits have not been cleared by the U.S. Food and Drug Administration for In Vitro Diagnostic use. The kits are for Research Use Only. Not for use in diagnostic procedures. The instructions 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. The brand and product names of the instruments are trademarks of their respective holders.

General Recommendations

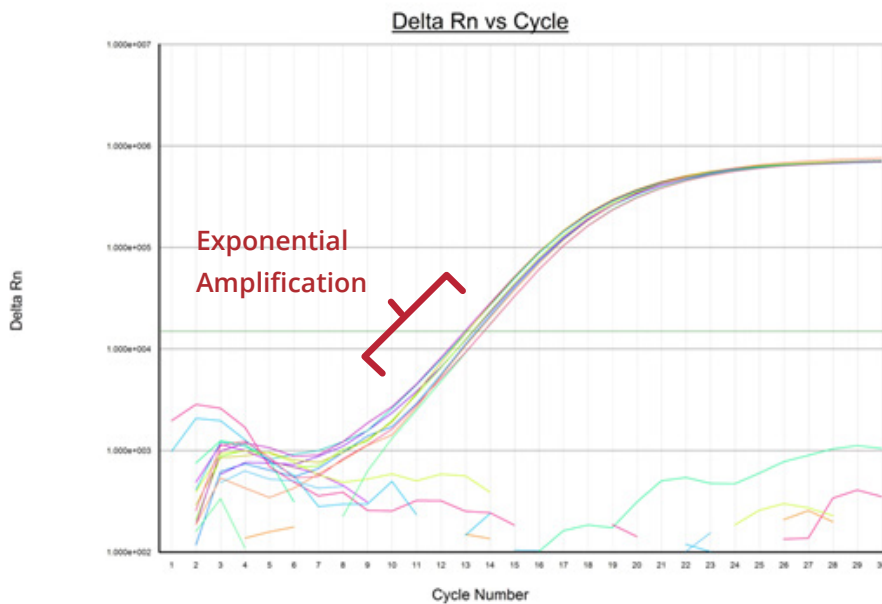
Instrument and Protocol Set-up: A template protocol can be made and reused for future assays to reduce instrument set-up time after the first run with each kit.

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 (Table 1 and Table 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).

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 (10-200ng/ μ L of bacterial DNA) and may be necessary to adjust 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.

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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 Table 2 and Table 4 for each respective target 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 your results.

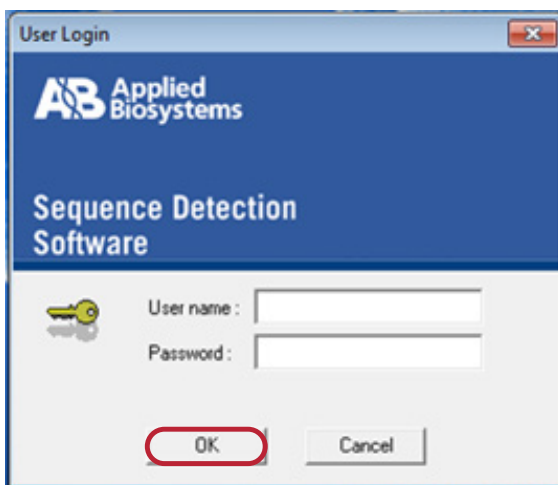
Specific set-up instructions are provided below.

Instrument Set-up

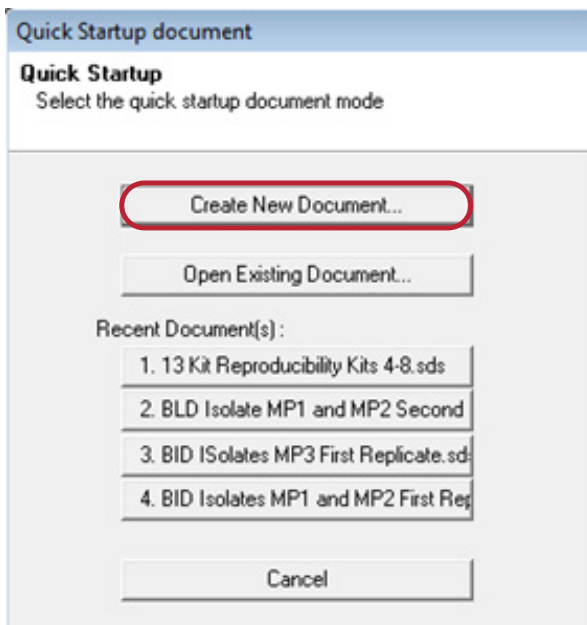
Open 7500 Fast System Software v 1.4.0.

Enter Credentials.

Click OK.



In the Quick Startup document window, select Create New Document.



Streck ARM-D® Kits: Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR System

On the New Document Wizard-Define Document window, make sure the 7500 Fast is selected for Run Mode.

Click Next.

New Document Wizard
Define Document
Select the assay, container, and template for the document, and enter the operator name and comments.

Assay: Standard Curve (Absolute Quantitation)
Container: 96-Well Clear
Template: Blank Document [Browse...]
Run Mode: Fast 7500
Operator: Admin
Comments: SDS v1.4.1
Plate Name: Plate1

< Back **Next >** Finish Cancel

On the New Document Wizard-Select Detectors window, click on New Detector and add each of the ARM-D Kit targets with the respective compatible reporter fluorophores (i.e., FAM, JOE (equivalent to HEX), Texas Red (equivalent to TEX615), and CY5 (equivalent to TYE665)). Refer to Table 1 and Table 3 for fluorophores conjugated to each respective target covered on the ampC and β -Lactamase kit respectively.

Click OK.

New Document Wizard
Select Detectors
Select the detectors you want to use in this document

Find: CMY

Detector Name	De
DHA-FAM	
DHA	
cbxm14sybr2	
CTX-M-15	
CTX-M-14 SYBR	
CTX-M-14	
CMY-TEX	
CMY-FAM	
CMY	
ACTB	Epi
ACC-HEX	

New Detector

Name: CMY
Description:
Reporter Dye: FAM
Quencher Dye: (none)
Color: ■
Notes:

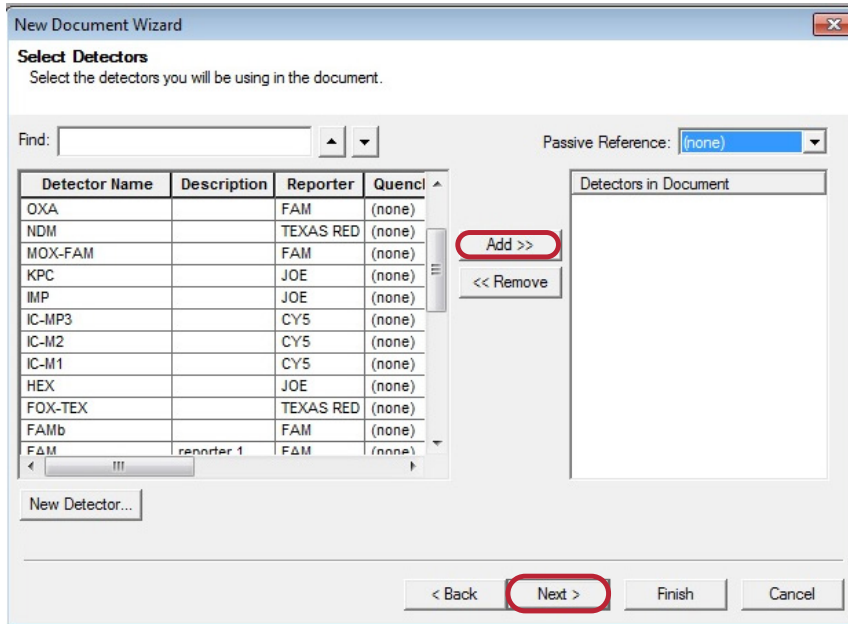
Create Another **OK** Cancel

< Back Next > Finish Cancel

Streck ARM-D® Kits: Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR System

After adding all the targets included within each kit, make sure they are included on the Document by Adding each one individually as shown below.

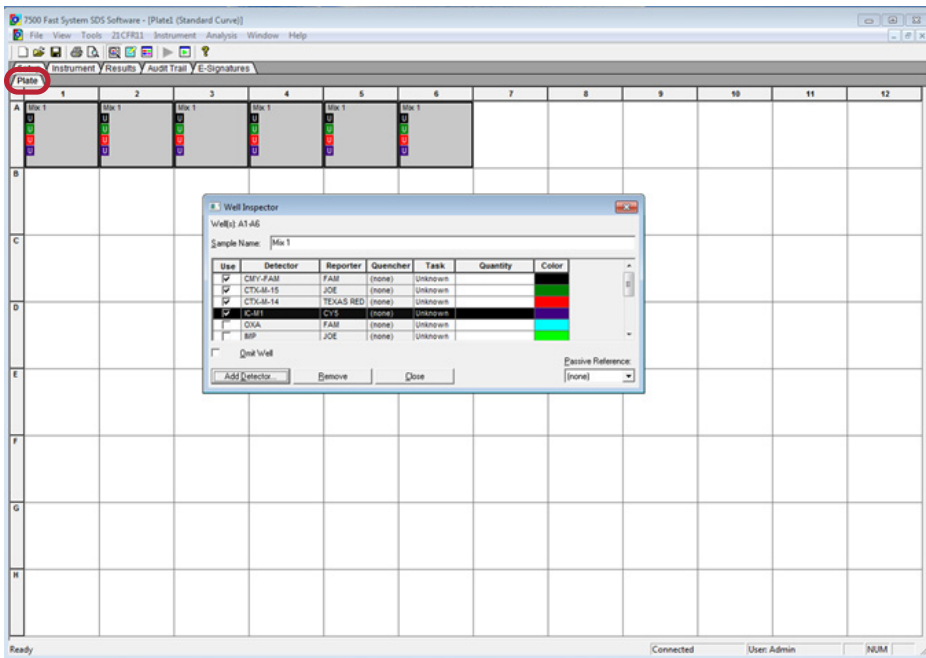
Click Next.



Important: Make sure that None is selected for the passive reference dye at the top of this window.

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On the **Plate** subtab, select wells and assign the appropriate samples, targets and tasks (Unknown, Positive Control or Negative Control). There should be no more than four targets in any single well.



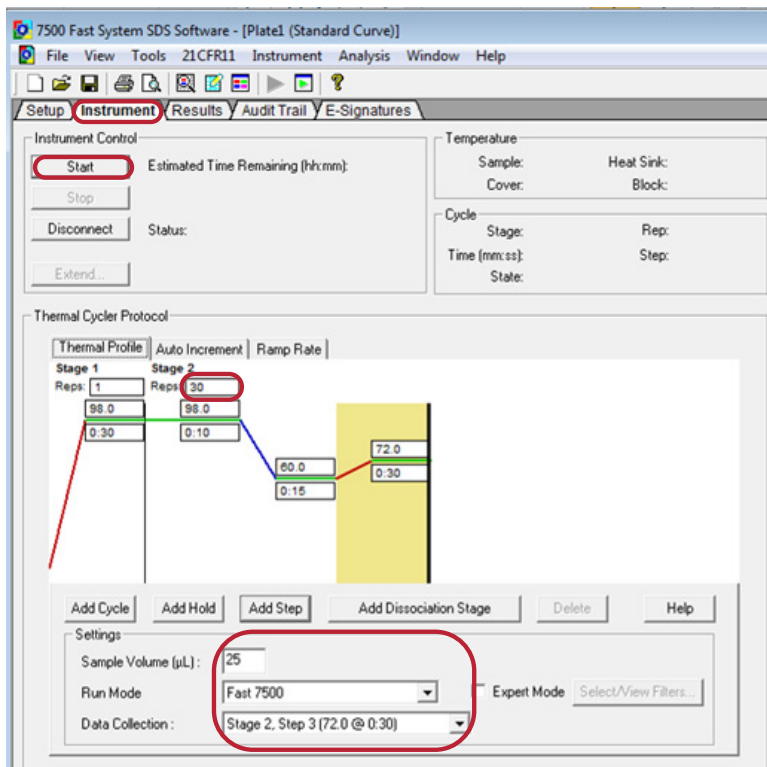
On the **Instrument** subtab, enter the Streck ARM-D Kit protocol as shown below.

Streck ARM-D Kit Cycling Protocol ABI 7500 Fast Dx

Hot Start 98 °C for 30 sec
 98 °C for 10 sec

30 cycles of 60 °C for 15 sec
 72 °C for 30 sec
 (Detection Step)

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

Change Reaction Volume to 25 µL.

Make sure Run Mode selected is Fast 7500.

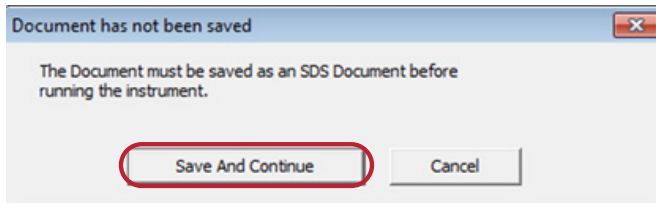
Change Number of Cycles to 3.

Make sure Data Collection On is active after the extension step.

Streck ARM-D® Kits: Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR System

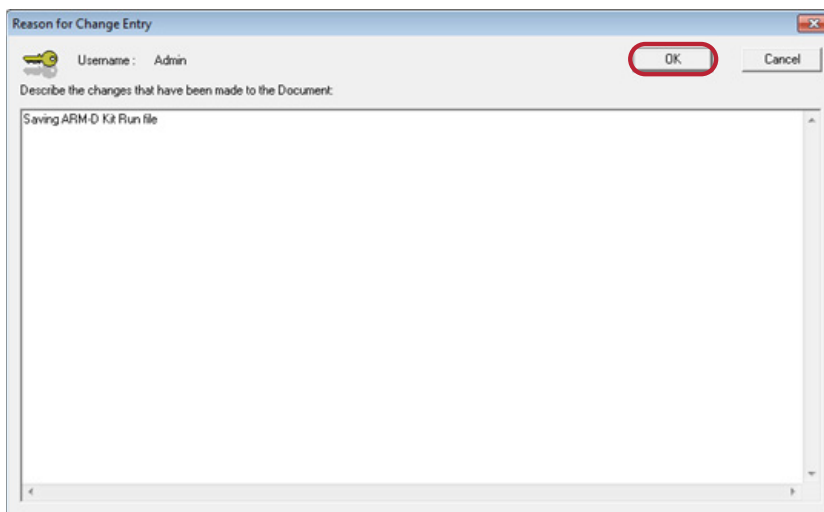
Click Start.

Click Save and Continue.



Enter reason for change entry as required by the software.

Click OK. The run should be complete within 45 minutes.



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

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

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 1.

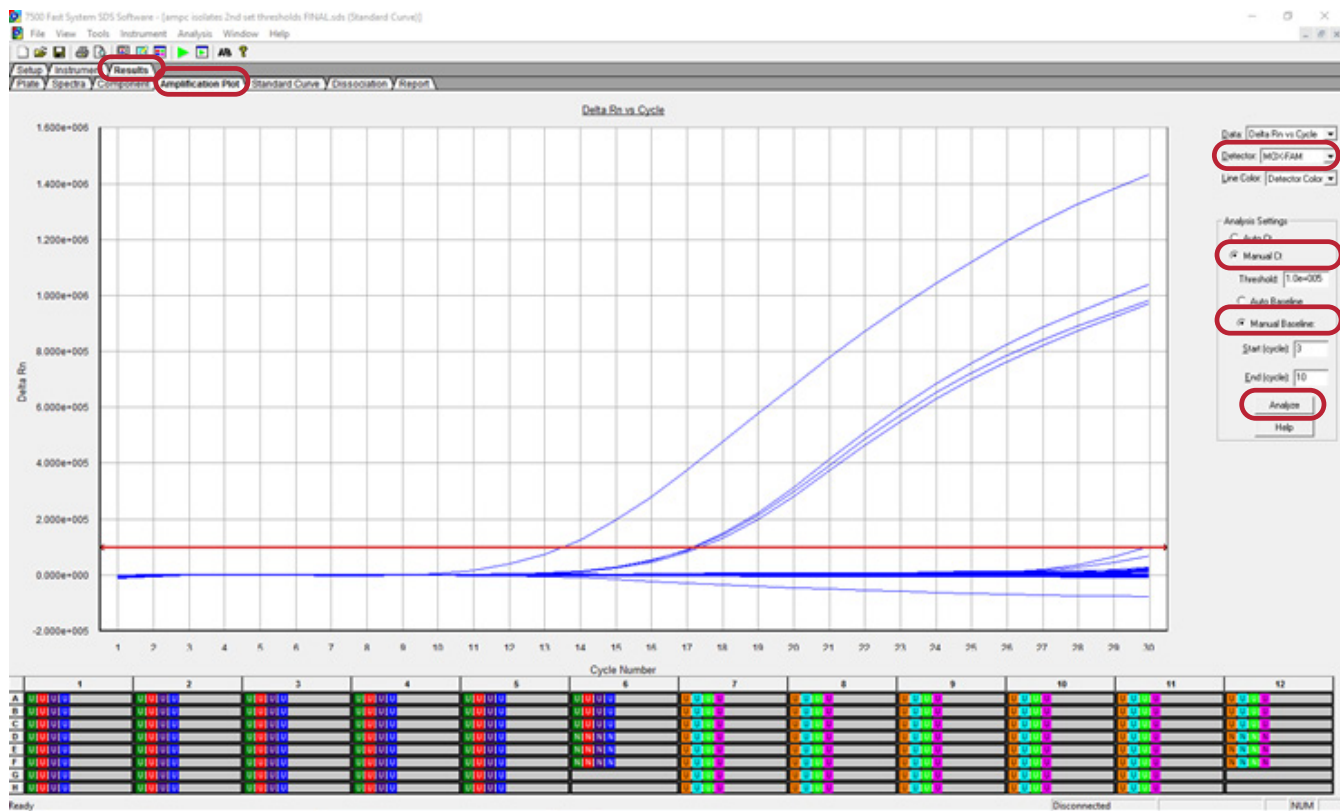
After opening a data file, click on the **Results** tab located at the top.

Click on the **Amplification Plot** tab.

Select each target individually on the top right corner. On the Analysis Settings deselect **Auto Ct** and **Auto Baseline**.

- Enter the corresponding threshold and baseline values specified in Table 1 (i.e., threshold value for MOX-ampC Kit is 100,000; Baseline Start Cycle is 3 and End Cycle is 10). Repeat for each target/fluorophore combination.

Click **Analyze**.



* 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.

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On the **Report** tab, record the Ct values for each target accordingly.

As mentioned above, if the Cq values fall outside of the 10-26 cycle range, data should be interpreted carefully prior to confirming a positive or negative result.

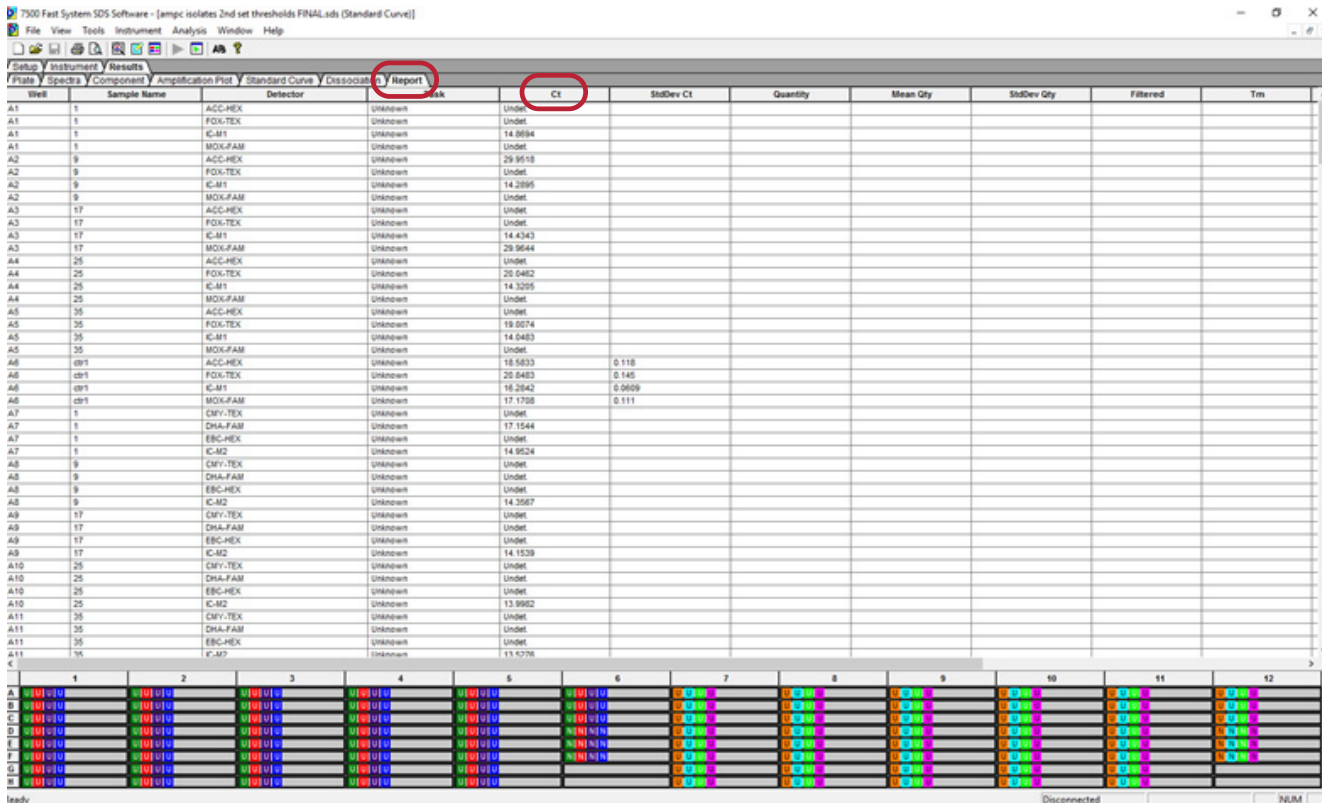


Table 1. Optical channels and threshold values determined during validation of the Streck ARM-D Kit, *ampC* (RUO) on the ABI 7500 Fast Dx Real-Time PCR System.

Master Mix	Target Gene Family	Fluorophore	Optical Channel	Threshold Values	Baseline Starts at Cycle	Baseline Ends at Cycle
PCR Mix 1	MOX	FAM	FAM	100,000	3	10
	ACC	HEX	JOE	35,000	3	10
	FOX	TEX615	Texas Red	70,000	3	10
	IC	TYE665	Cy5	32,000	3	10
PCR Mix 2	DHA	FAM	FAM	60,000	3	10
	EBC	HEX	JOE	80,000	3	10
	CMY-2	TEX615	Texas Red	200,000	3	10
	IC	TYE665	Cy5	32,000	3	10

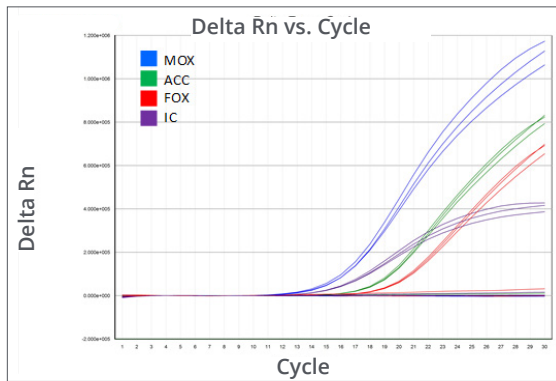
Streck ARM-D® Kits: Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR System

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, *ampC* (RUO) is shown in Figure 1. Although Cq values for amplification plot of unknown samples may vary from sample to sample, representative amplification data of *ampC*-positive clinical isolates is shown in Figure 2. Refer to the [Data Interpretation](#) section for specific guidelines on interpreting unknown sample data.

Control Mix 1



Control Mix 2

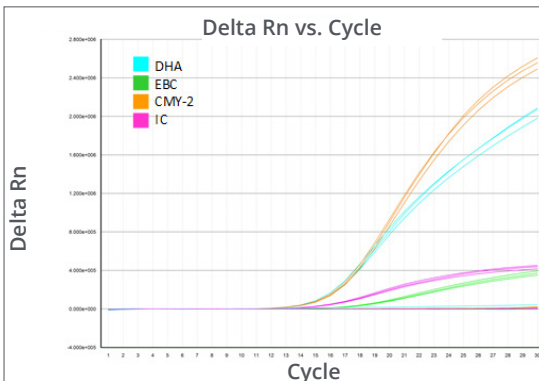
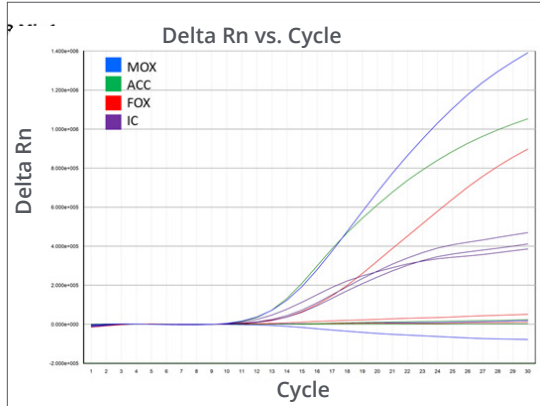


Figure 1: Multiplex real-time PCR amplification data of positive DNA Control Mixes of Streck ARM-D Kit, *ampC* on the ABI 7500 Fast Dx Real-Time PCR System.

PCR Mix 1



PCR Mix 2

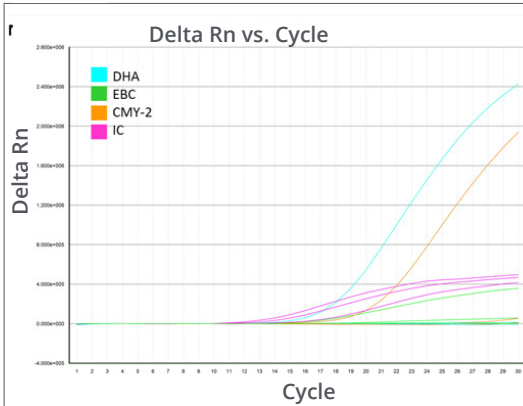


Figure 2: Amplification of *ampC*-positive clinical isolates using Streck ARM-D Kit, *ampC* (RUO). Data shows the amplification of six DNA samples that are positive for one of the respective *ampC* targets detected with the kit on the ABI 7500 Fast Dx Real-Time PCR System. The IC was detected in each sample.

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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 ABI 7500 Fast Dx Real-Time PCR System fell within the range specified in Table 2. **These values should only 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* (RUO).

Control Mix	Target Gene Family (Fluorophore)	C _q Value Range
Mix 1	MOX (FAM)	17 ± 3
	ACC (HEX)	19 ± 3
	FOX (TEX615)	21 ± 3
	IC (TYE665)	16 ± 3
Mix 2	DHA (FAM)	15 ± 3
	EBC (HEX)	20 ± 3
	CMY-2 (TEX615)	17 ± 3
	IC (TYE665)	16 ± 3

Cq Values – Unknown Samples

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

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

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.

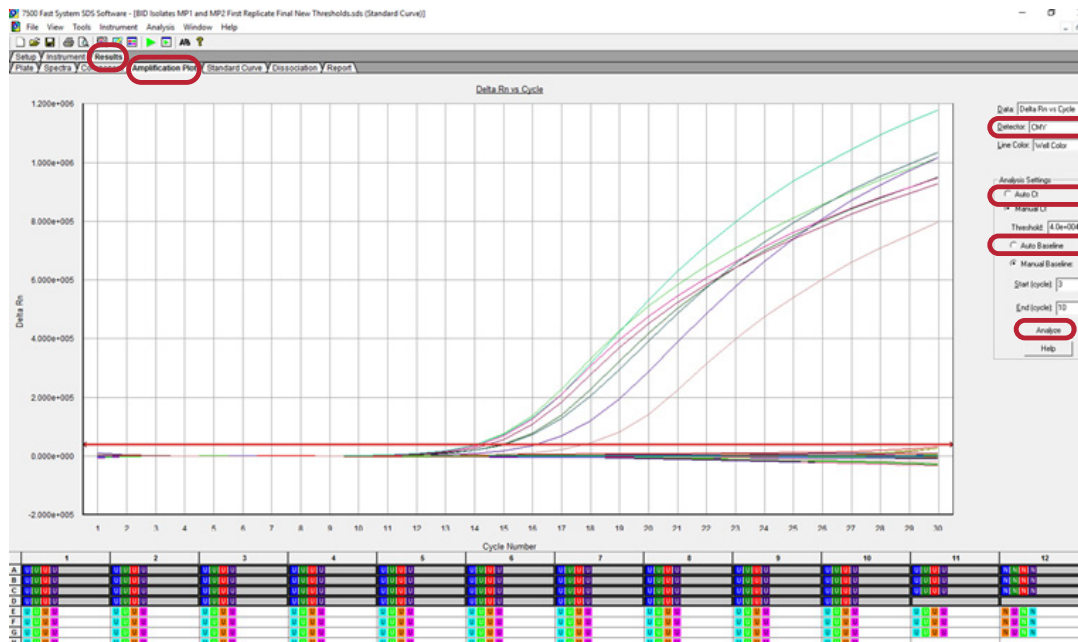
After opening a data file, click on the Results tab located at the top.

Click on the **Amplification Plot** tab.

Select each target individually on the top right corner. On the Analysis Settings deselect **Auto Ct** and **Auto Baseline**.

- Enter the corresponding threshold and baseline values specified in Table 3 (i.e., threshold value for CMY- β -lactamase kit is 40,000; Baseline Start Cycle is 3 and End Cycle is 10). Repeat for each target/fluorophore combination.

Click Analyze.



** 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.

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On the **Report** tab record the Ct values for each target accordingly.

As mentioned above, 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.

The screenshot displays the 'Report' tab in the 7500 Fast System SDS Software. The main table lists the following columns: Well, Sample Name, Detector, Target, Ct, StdDev Ct, Quantity, Mean Qty, StdDev Qty, Filtered, and Tm. The 'Report' and 'Ct' columns are circled in red. Below the table is a grid of colored squares representing PCR wells, with columns labeled 1 through 12 and rows labeled A through H. Each well contains a small icon representing the detected target.

Well	Sample Name	Detector	Target	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm
A1	1	CMV	Unknown	Under						
A1	1	CTV-M-15	Unknown	Under						
A1	1	CTV-M-14	Unknown	Under						
A1	1	IC-M1	Unknown	13.6207						
A2	2	CMV	Unknown	Under						
A2	2	CTV-M-15	Unknown	18.3295						
A2	2	CTV-M-14	Unknown	Under						
A2	2	IC-M1	Unknown	13.6267						
A3	3	CMV	Unknown	Under						
A3	3	CTV-M-15	Unknown	Under						
A3	3	CTV-M-14	Unknown	Under						
A3	3	IC-M1	Unknown	14.7247						
A4	4	CMV	Unknown	Under						
A4	4	CTV-M-15	Unknown	Under						
A4	4	CTV-M-14	Unknown	Under						
A4	4	IC-M1	Unknown	14.6467						
A5	5	CMV	Unknown	Under						
A5	5	CTV-M-15	Unknown	Under						
A5	5	CTV-M-14	Unknown	Under						
A5	5	IC-M1	Unknown	13.1894						
A6	6	CMV	Unknown	Under						
A6	6	CTV-M-15	Unknown	17.655						
A6	6	CTV-M-14	Unknown	Under						
A6	6	IC-M1	Unknown	12.7773						
A7	7	CMV	Unknown	Under						
A7	7	CTV-M-15	Unknown	Under						
A7	7	CTV-M-14	Unknown	Under						
A7	7	IC-M1	Unknown	13.0638						
A8	8	CMV	Unknown	Under						
A8	8	CTV-M-15	Unknown	Under						
A8	8	CTV-M-14	Unknown	18.6050						
A8	8	IC-M1	Unknown	17.0789						
A9	9	CMV	Unknown	Under						
A9	9	CTV-M-15	Unknown	Under						
A9	9	CTV-M-14	Unknown	Under						
A9	9	IC-M1	Unknown	12.8135						
A10	10	CMV	Unknown	Under						
A10	10	CTV-M-15	Unknown	Under						
A10	10	CTV-M-14	Unknown	Under						
A10	10	IC-M1	Unknown	14.2897						
A11	0B1	CMV	Unknown	Under						
A11	0B1	CTV-M-15	Unknown	16.2318						
A11	0B1	CTV-M-14	Unknown	14.4218						
A11	0B1	IC-M1	Unknown	16.1071						

Streck ARM-D® Kits: Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR System

Table 3. Optical channels and threshold values determined during validation of the Streck ARM-D Kit, β -Lactamase (RUO) on the ABI 7500 Fast Dx 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	40,000	3	10
	CTX-M-15	HEX	JOE	40,000	3	10
	CTX-M-14	TEX615	Texas Red	40,000	3	10
	IC	TYE665	Cy5	40,000	3	10
PCR Mix 2	OXA-48	FAM	FAM	40,000	3	10
	IMP	HEX	JOE	50,000	3	10
	VIM	TEX615	Texas Red	50,000	3	10
	IC	TYE665	Cy5	40,000	3	10
PCR Mix 3	DHA	FAM	FAM	40,000	3	10
	KPC	HEX	JOE	40,000	3	10
	NDM	TEX615	Texas Red	40,000	3	10
	IC	TYE665	Cy5	40,000	3	10

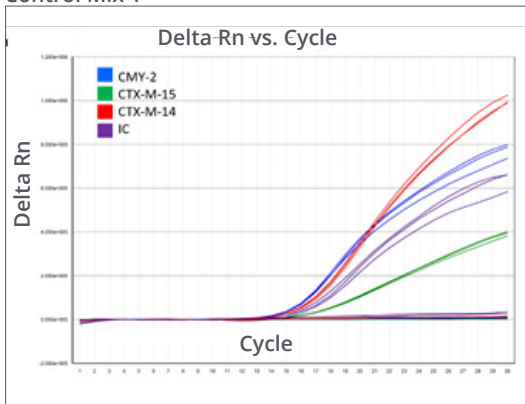
Amplification Curve Data

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

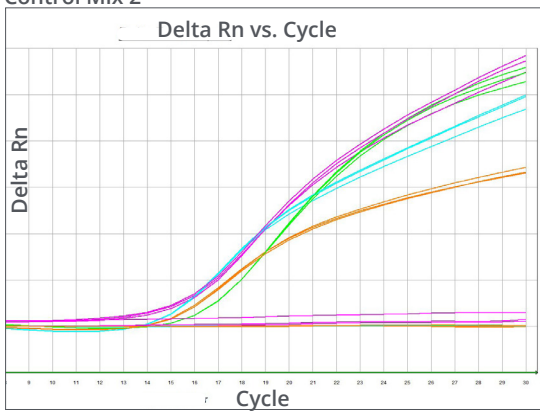
Characteristic amplification data for positive control targets detected with Streck ARM-D Kit, β -Lactamase (RUO) is shown in Figure 3. Although Cq values for amplification plot of unknown samples may vary from sample to sample, representative amplification data of β -lactamase-positive clinical isolates is shown in Figure 4. Refer to the [Data Interpretation](#) section for specific guidelines on interpreting unknown sample data.

Streack ARM-D® Kits: Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR System

Control Mix 1



Control Mix 2



Control Mix 3

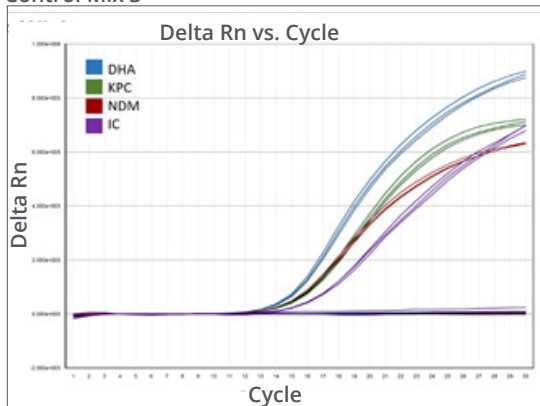
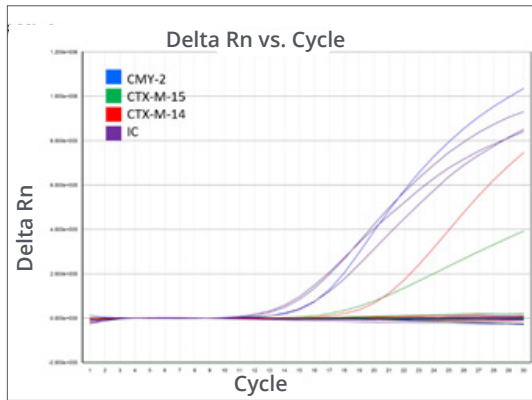


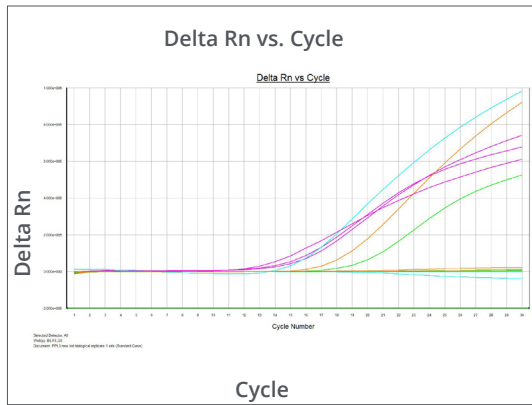
Figure 3. Multiplex real-time PCR amplification data of positive DNA Control Mixes of Streack ARM-D Kit, β -Lactamase (RUO) on the ABI 7500 Fast Dx Real-Time PCR System.

Streck ARM-D® Kits: Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR System

PCR Mix 1



PCR Mix 2



PCR Mix 3

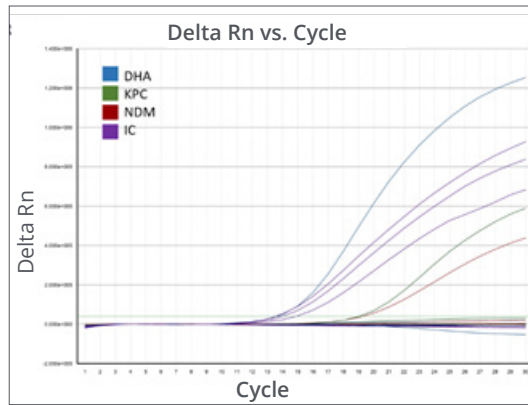


Figure 4. Amplification of β -lactamase-positive clinical isolates using Streck ARM-D Kit, β -Lactamase (RUO). Data shows the amplification of nine DNA samples that are positive for one of the respective β -Lactamase targets detected with the kit on the ABI 7500 Fast Dx Real-Time PCR System. The IC was detected in each sample.

Streck ARM-D® Kits: Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR System

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 ABI 7500 Fast Dx 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.

Table 4. Cq values for positive control targets determined during validation of the Streck ARM-D Kit, β -Lactamase (RUO).

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

Cq Values – Unknown samples

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

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, JOE, TEXAS RED	Cq IC (CY5)	Interpretation
$\leq 26^*$	10 to 20**	Positive Sample
NA	10 to 20**	Negative Sample
NA or > 26	NA or > 26	Invalid

** Typical Cq values obtained for 10-200ng/ μ l purified DNA samples

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

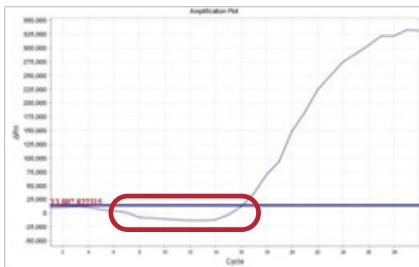
Negative Sample: If no Cq value is detected in the FAM, JOE, and Texas Red channels for unknown samples, confirm sample was added to the reactions by verifying positive amplification of the internal control (IC) for Gram-negative bacteria in the Cy5 channel (Cq = 10 to 20). If IC (Cy5) is amplified and no amplification is detected in FAM, JOE, 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.

Invalid/Flag for Further Investigation: If amplification of an unknown sample in the FAM, JOE, Texas Red, and Cy5 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.

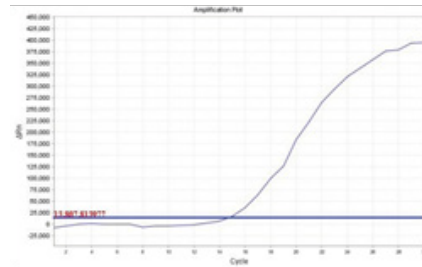
Troubleshooting

- 1. Amplification is not observed for any sample after the PCR protocol is complete:** Verify that ROX is not selected as a passive reference dye in the Setup window. Refer to instrument set-up 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.
- 2. Incorrect baseline settings:** For some samples, automatic baseline settings that are erroneously assigned may cause false positive or false negative values. Usually this is noticeable in the amplification plots, as in the example below:

Incorrect baseline

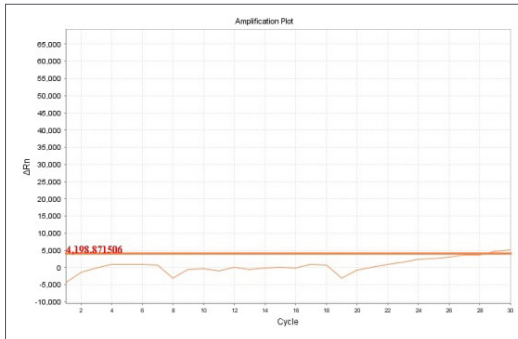


Correct baseline



Streck ARM-D® Kits: Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR System

3. **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, signal noise resulted in a Cq value of 28.5, indicating the potential presence of the specific target. However, on examination of the data, it becomes apparent that the increase in fluorescence intensity is due to artifacts, and the sample should be considered negative for that target. In this case, a slightly higher threshold would have resulted in the correct interpretation (no amplification).



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.