

Streck ARM-D[®] Kits

Data Acquisition and Analysis Guide

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

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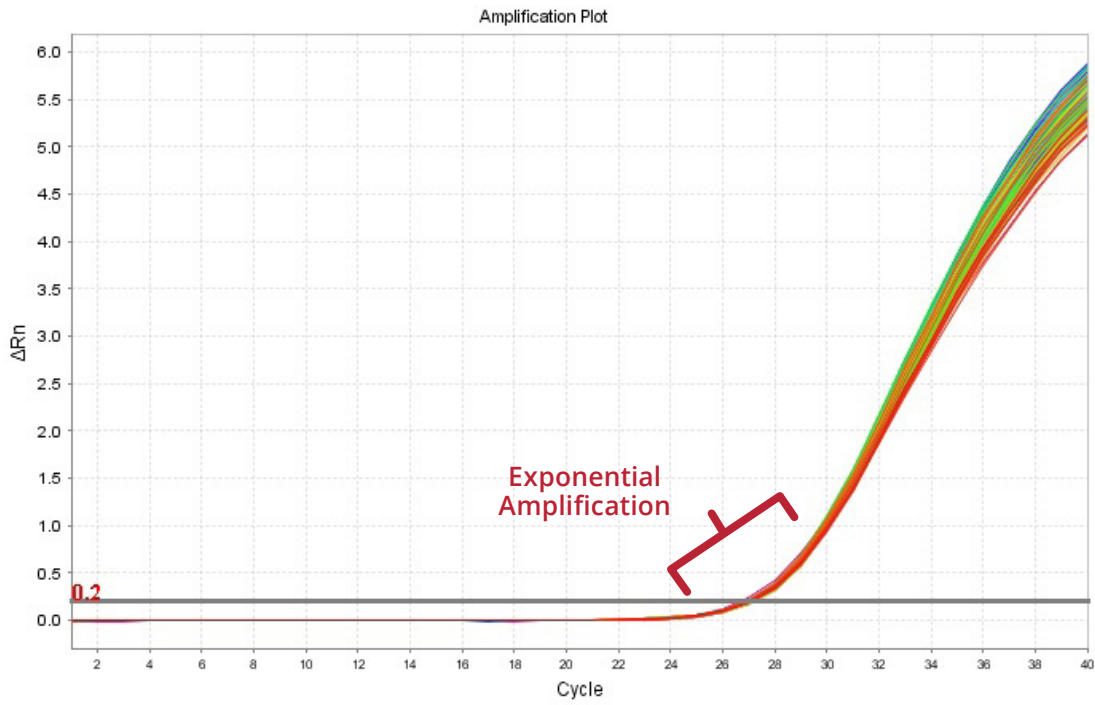
This guide is intended to be used as an ARM-D Kit-specific supplement for the Instructions For Use (IFU) document included with each kit. The kits have not been cleared by the U.S. Food and Drug Administration for In Vitro Diagnostic use. The kits are for Research Use Only. Not for use in diagnostic 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.

General Recommendations

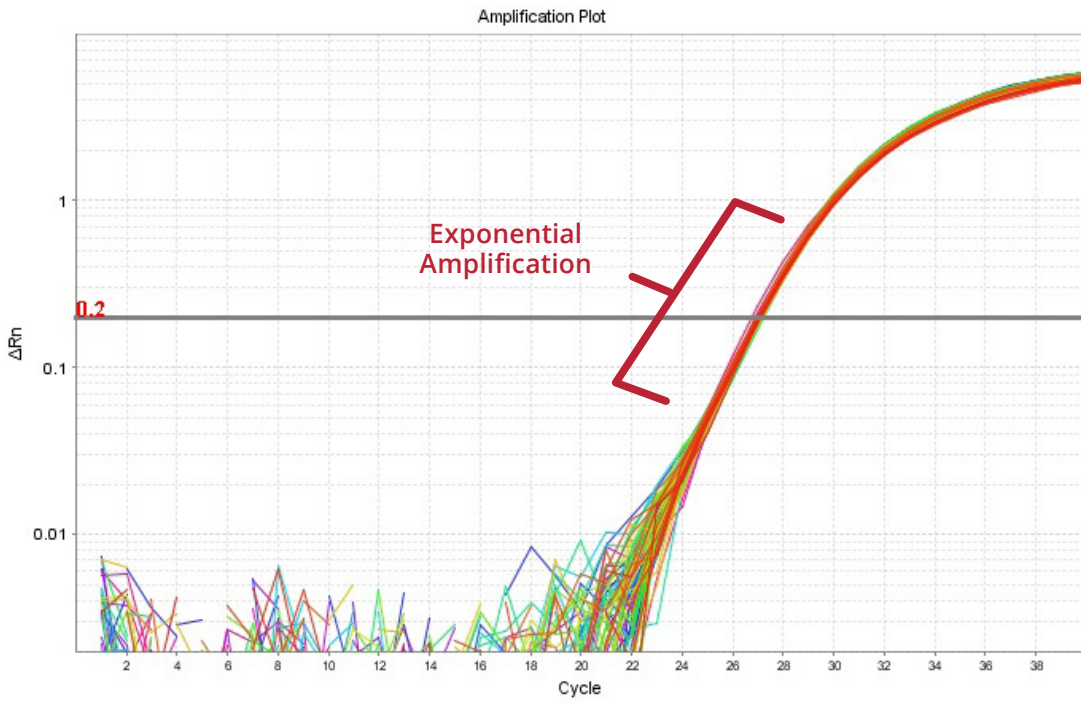
Instrument and Protocol Set-up: A template protocol can be made and reused for future assays to reduce instrument set-up time after the first run with each kit.

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 next page).

Linear Scale View



Log Scale View



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

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, 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.

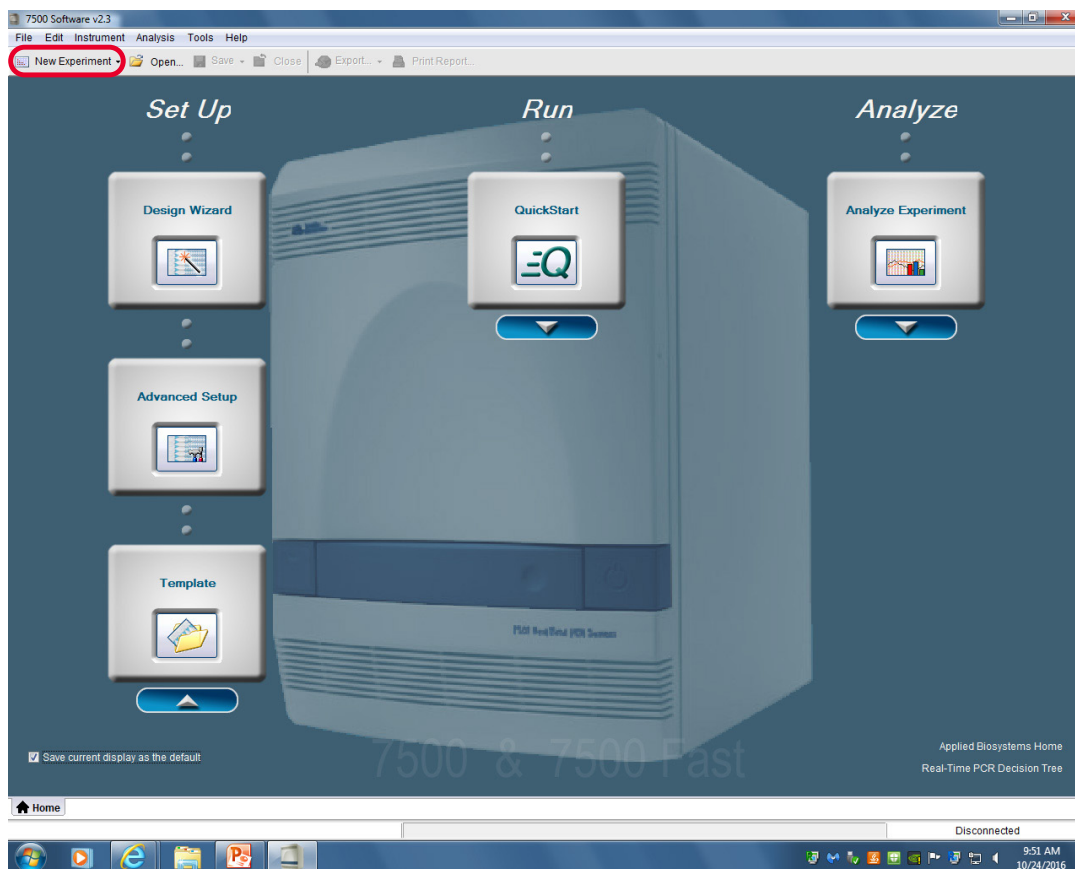
Assay Performance: It is expected that Cq values for positive controls and unknown samples should demonstrate amplification between cycles 10 and 26. Cq values determined for positive controls during internal validation are provided in Tables 2 and 4 for each respective kit as a guideline. Due to variations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

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

Instrument Set-up

Open the ABI 7500 Software.

Click on **New Experiment**.



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

- Instrument type: 7500 Fast (96 Wells).
- Type of experiment: Quantitation-Standard Curve.
- Reagents: TaqMan Reagents.
- Ramp speed: Fast (~40 minutes to complete run).

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

7500 Software v2.3

File Edit Instrument Analysis Tools Help

New Experiment Open Save Close Export Print Report

Experiment Menu << Experiment: Untitled Type: Standard Curve Reagents: TaqMan® Reagents START RUN ?

Setup

Experiment Properties

Plate Setup

Run Method

Reaction Setup

Materials List

Run

Analysis

Experiment Properties

How do you want to identify this experiment?

* Experiment Name: Untitled

Barcode (Optional):

User Name (Optional):

Comments (Optional):

* Which instrument are you using to run the experiment?

7500 (96 Wells) ✓ 7500 Fast (96 Wells)

Set up, run, and analyze an experiment using a fast cycling 5-color, 96-well system.

* What type of experiment do you want to set up?

✓ Quantitation - Standard Curve Quantitation - Relative Standard Curve Quantitation - Comparative Ct ($\Delta\Delta C_t$)

Melt Curve Genotyping Presence/Absence

Use standards to determine the absolute quantity of target nucleic acid sequence in samples.

* Which reagents do you want to use to detect the target sequence?

✓ TaqMan® Reagents SYBR® Green Reagents Other

The PCR reactions contain primers designed to amplify the target sequence and a TaqMan® probe designed to detect amplification of the target sequence.

* Which ramp speed do you want to use in the instrument run?

Standard (~ 2 hours to complete a run) ✓ Fast (~ 40 minutes to complete a run)

For optimal results with the Fast ramp speed, Applied Biosystems recommends using Fast reagents for your PCR reactions.

Home Untitled x

Monday, October 24, 2016 9:53 AM 10/24/2016

Note: The Streck ARM-D Kits were validated with the 7500 Fast (96-wells) block configuration.

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

Add sample names and/or identify targets for each PCR Mix by their respective fluorophore/optical channel combinations as described below in Table 1 for the Streck ARM-D Kit, *ampC* (RUO) and Table 3 for the Streck ARM-D Kit, β -Lactamase (RUO) (See Data Analysis section).

The screenshot shows the '7500 Software v2.3' interface. The 'Experiment Menu' on the left has 'Setup' selected, and 'Plate Setup' is highlighted. The main window is titled 'Experiment: Untitled' and 'Type: Standard Curve'. The 'Define Targets and Samples' tab is active, showing instructions to define targets and samples. The 'Define Targets' table is as follows:

Target Name	Reporter	Quencher	Color
MOX	FAM	NFQ-MGB	Blue
ACC	JOE	NFQ-MGB	Green
FOX	TEXAS RED	NFQ-MGB	Red
ICM1	CY5	NFQ-MGB	Purple

The 'Define Samples' table is as follows:

Sample Name	Color
Control	Blue
NTC	Green

The 'Assign Targets and Samples' button is located at the bottom right of the 'Define Targets and Samples' section.

After completing the Define Target and Samples definitions, click on the **Assign Targets and Samples** tab at the bottom of the screen, or select the tab by the same name.

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

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.

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

7500 Software v2.3

File Edit Instrument Analysis Tools Help

New Experiment Open Save Close Export Print Report

Experiment Menu << Experiment: Untitled Type: Standard Curve Reagents: TaqMan® Reagents START RUN ?

Setup

Experiment Properties

Plate Setup

Run Method

Reaction Setup

Materials List

Run

Analysis

Define Targets and Samples **Assign Targets and Samples**

Instructions: To set up standards: Click "Define and Set Up Standards." To set up unknowns: Select wells, assign target(s), select "U" (Unknown) as the task for each target assignment, then assign a sample. To set up negative controls: Select wells, assign target(s), then select "N" (Negative Control) as the task for each target assignment.

Assign target(s) to the selected wells.

Assign	Target	Task	Quantity
<input checked="" type="checkbox"/>	MOX	<input type="checkbox"/> <input checked="" type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
<input checked="" type="checkbox"/>	ACC	<input type="checkbox"/> <input checked="" type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
<input checked="" type="checkbox"/>	FOX	<input type="checkbox"/> <input checked="" type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	

* Mixed Unknown Standard Negative Control

Define and Set Up Standards

Assign sample(s) to the selected wells.

Assign	Sample
<input checked="" type="checkbox"/>	Control
<input type="checkbox"/>	NTC

Assign sample(s) of selected well(s) to biological

Assign	Biological Group
--------	------------------

Select the dye to use as the passive reference.

None

View Plate Layout View Well Table

Select Wells With: - Select Item - - Select Item -

Show in Wells View Legend

	1	2	3	4	5	6	7	8	9	10	11	12
A		<input checked="" type="checkbox"/> FOX <input checked="" type="checkbox"/> ic		<input checked="" type="checkbox"/> FOX <input checked="" type="checkbox"/> ic								
B		<input checked="" type="checkbox"/> FOX <input checked="" type="checkbox"/> ic		<input checked="" type="checkbox"/> FOX <input checked="" type="checkbox"/> ic								
C		<input checked="" type="checkbox"/> FOX <input checked="" type="checkbox"/> ic		<input checked="" type="checkbox"/> FOX <input checked="" type="checkbox"/> ic								
D												
E												
F												
G												
H												

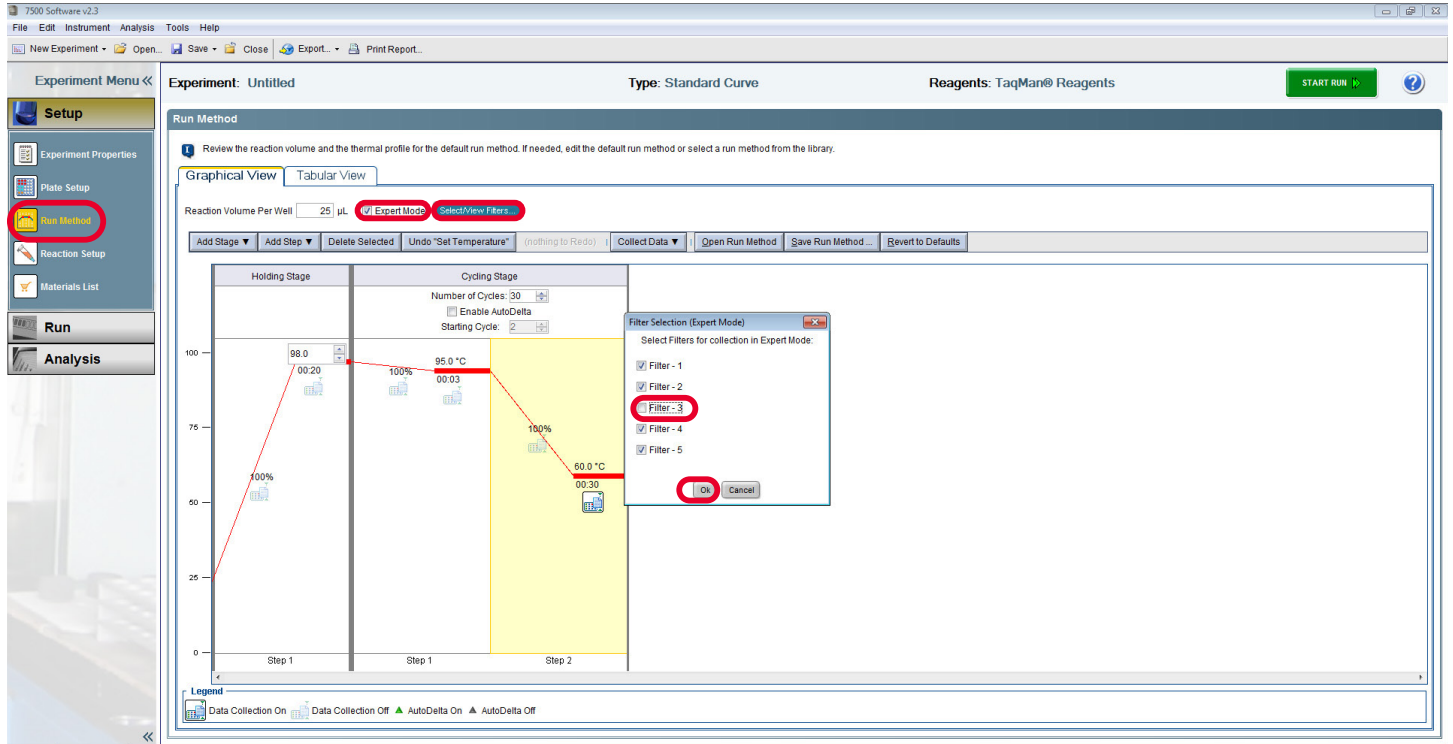
Wells: 6 Unknown 0 Standard 0 Negative Control 90 Empty

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

On the Run Method tab in the Setup window, select the **Expert Mode** box and then click the **Select/View Filters** button.

In the **Filter Selection** window, deselect Filter-3 (TAMRA, NED, Cy3 channel).

Click **OK**.



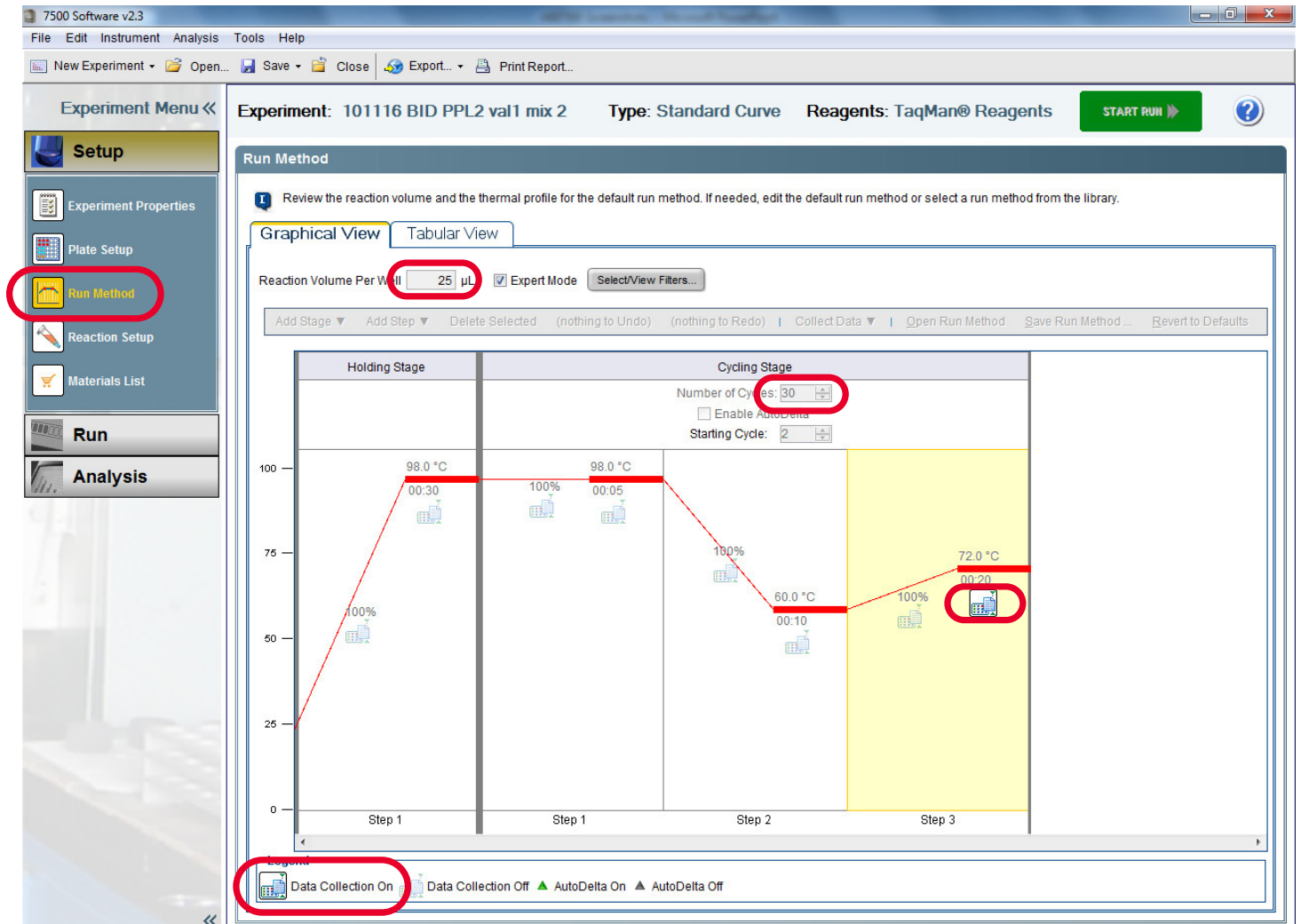
Enter the Streck ARM-D Kit protocol as shown below. Note that the PCR cycling protocol is the same for both Streck ARM-D *ampC* (RUO) and β -Lactamase (RUO) Kits.

Streck ARM-D Kit Cycling Protocol	
Hot Start	98 °C for 30 sec
30 cycles of	98 °C for 10 sec
	60 °C for 15 sec
	72 °C for 20 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 μ L.
- Change Number of Cycles to 30.
- Make sure Data Collection On is active after the extension step.



Click **Start Run**. The run should be complete within 45 minutes.

Note: If Filter-3 is not de-selected as described above, an error message will appear in the screen prior to running the PCR protocol. The error message will indicate that holding time in the last cycling step must be over 20 seconds.

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.

* 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 (ABI) 7500 Fast Real-Time PCR System

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.

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

The screenshot displays the 7500 Software v2.3 interface. The main window is titled "7500 Software v2.3" and contains a menu bar (File, Edit, Instrument, Analysis, Tools, Help) and a toolbar with options like "New Experiment", "Open...", "Save", "Close", "Export...", and "Print Report...".

The interface is divided into several sections:

- Experiment Menu:** Includes "Setup", "Run", and "Analysis" tabs. Under "Analysis", there are sub-options: "Amplification Plot", "Standard Curve", "Multicomponent Plot", "Raw Data Plot", "QC Summary", and "Multiple Plots View".
- Amplification Plot:** Shows a graph of ΔRn vs Cycle. The plot is titled "MOX". The y-axis ranges from -50,000 to 700,000, and the x-axis ranges from 2 to 30. A legend indicates ACC (green), FOX (red), IC (purple), and MOX (blue). The plot shows several curves starting at a baseline around 50,000 and rising sharply after cycle 10. A red horizontal line is drawn at approximately 50,000, with the value "51.888.872822" displayed below it.
- View Plate Layout:** Shows a 96-well plate layout with columns 1-12 and rows A-H. The wells are color-coded: red for FOX, purple for IC, and blue for MOX. The wells are labeled with "U" (Unknown), "F" (FOX), "Ct" (Cycle threshold), and "Ur" (Unknown reaction). The summary at the bottom of the plate layout reads: "Wells: 43 Unknown, 0 Standard, 0 Negative Control, 53 Empty".
- Options:** Includes "Target: All", "Threshold: Auto", "Auto Baseline", "Show: Threshold", "Baseline Start: Well", "Target", "Baseline End: Well", and "Save current settings as the default".

The bottom status bar shows "Analysis Summary: Total Wells in Plate: 96, Wells Set Up: 43, Wells Omitted Manually: 0, Wells Flagged: 0, Wells Omitted by Analysis: 0, Samples Used: 43, Targets Used: 4".

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On the Ct Settings tab in the Analysis Settings window, deselect the following: Use Default Settings; Automatic Threshold and Automatic Baseline.

Enter the corresponding threshold and baseline values specified in Table 1 (i.e., threshold value for MOX and FOX is 25,000; Baseline Start Cycle is 3 and End Cycle is 10). Repeat for each target/fluorophore combination.

Click Apply Analysis Settings button.

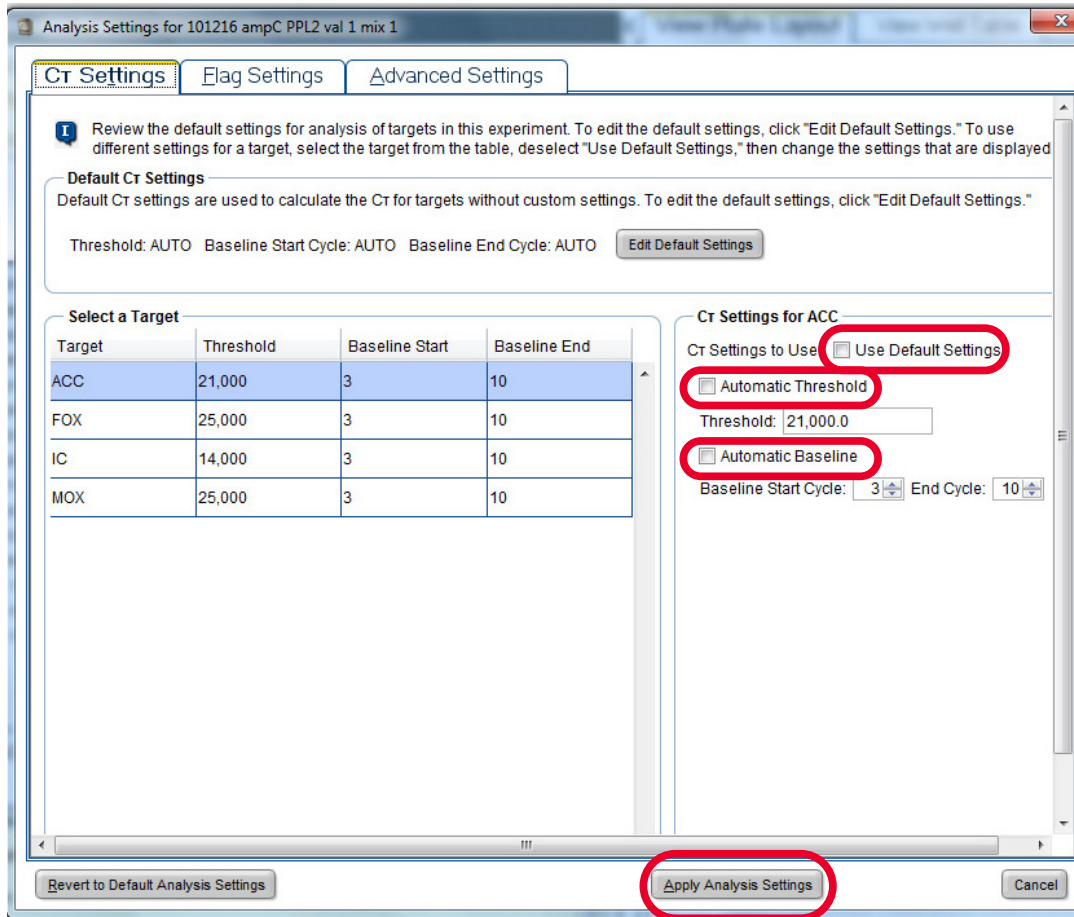


Table 1. Optical channels and threshold values determined during validation of the Streck ARM-D Kit, *ampC* on the ABI 7500 Fast Real-Time PCR System.

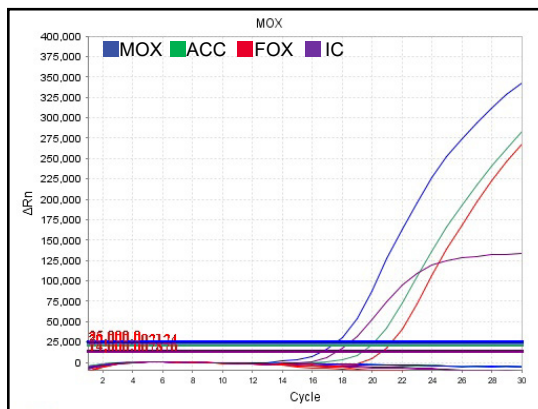
Master Mix	Target Gene Family	Fluorophore	Optical Channel	Threshold Values	Baseline Starts at Cycle	Baseline Ends at Cycle
PCR Mix 1	MOX	FAM	FAM	25,000	3	10
	ACC	HEX	JOE	21,000	3	10
	FOX	TEX615	Texas Red	25,000	3	10
	IC	TYE665	Cy5	14,000	3	10
PCR Mix 2	DHA	FAM	FAM	40,000	3	10
	EBC	HEX	JOE	38,000	3	10
	CMY-2	TEX615	Texas Red	90,000	3	10
	IC	TYE665	Cy5	14,000	3	10

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

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* (RUO) 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.

Control Mix 1



Control Mix 2

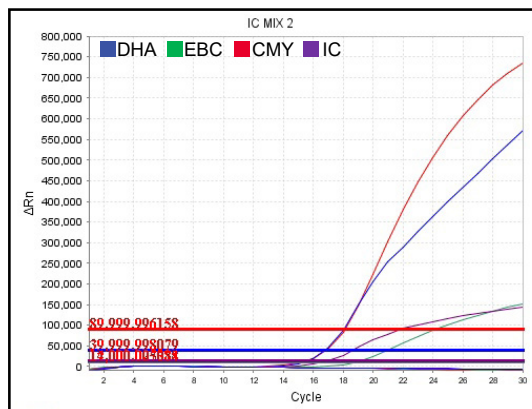
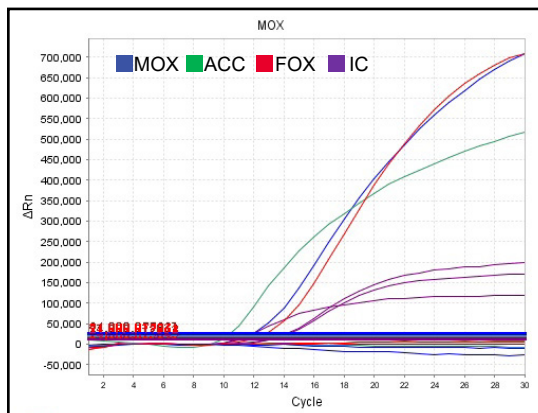


Figure 1. Multiplex real-time PCR amplification data of positive DNA Control Mixes for the Streck ARM-D Kit, *ampC*, on the ABI 7500 Fast Real-Time PCR System.

PCR Mix 1



PCR Mix 2

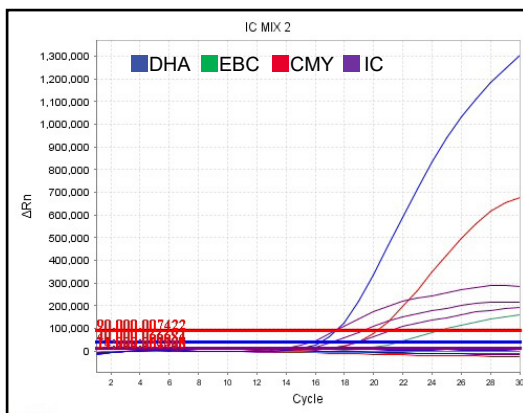


Figure 2. Amplification of plasmid-mediated *ampC*-positive clinical isolates using Streck ARM-D Kit, *ampC*. The data above shows amplification of six clinical isolates that are positive for each respective *ampC* target detected by the kit. The IC (purple lines) was detected in each sample.

Streck ARM-D® Kits: Applied Biosystems (ABI) 7500 Fast 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 ABI 7500 Fast 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)	19 ± 3
	FOX (TEX615)	20 ± 3
	IC (TYE665)	16 ± 3
Mix 2	DHA (FAM)	16 ± 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* 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 (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.

** 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 (ABI) 7500 Fast Real-Time PCR System

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** tab located at the top right hand corner of the analysis screen.

7500 Software v2.3

File Edit Instrument Analysis Tools Help

New Experiment Open... Save Close Export... Print Report...

Experiment Menu << Experiment: 101116 BID PPL2 val1 mix 1 Type: Standard Curve Reagents: TaqMan® Reagents Analyze Analysis Settings ?

Setup

Run

Analysis

Amplification Plot

Standard Curve

Multicomponent Plot

Raw Data Plot

QC Summary

Multiple Plots View

Amplification Plot

Plot Settings

Plot Type: ΔRn vs Cycle Graph Type: Linear Plot Color: Target

Save current settings as the default

IC MIX 1

ΔRn

Cycle

15,000.002923

Legend

CTX-M-14 BID IC BID CMY-BID CTX-M-15 BID

Options

Target: All Threshold: Auto Auto Baseline

Show: Threshold Baseline Start: Well Target Baseline End: Well

Save current settings as the default

View Plate Layout View Well Table

Select Wells With: -Select Item - -Select Item -

Show in Wells View Legend

Wells: 43 Unknown 0 Standard 0 Negative Control 53 Empty

Analysis Summary: Total Wells in Plate: 96 Wells Set Up: 43 Wells Omitted Manually: 0 Wells Flagged: 0 Wells Omitted by Analysis: 0 Samples Used: 43 Targets Used: 4

Home Untitled x 101216 ampC P...al 1 mix 1.ed.s x 101216 ampC P...al 1 mix 2.ed.s x 101116 BID PP...val1 mix 1.ed.s x

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

On the Ct Settings tab in the Analysis Settings window, deselect the following: Use Default Settings; Automatic Threshold and Automatic Baseline.

Enter the corresponding threshold and baseline values specified in Table 1 (i.e., threshold value for CMY, CTX-M-14, CTX-M-15, and IC is 15,000; Baseline Start Cycle is 3 and End Cycle is 10). Repeat for each target/flouorophore combination.

Click Apply Analysis Settings button.

Analysis Settings for 101116 BID PPL2 val1 mix 1

Ct Settings | Flag Settings | Advanced Settings

Review the default settings for analysis of targets in this experiment. To edit the default settings, click "Edit Default Settings". To apply different settings for a target, select the target from the table, deselect "Use Default Settings," then change the settings that you want to use.

Default Ct Settings
Default Ct settings are used to calculate the Ct for targets without custom settings. To edit the default settings, click "Edit Default Settings".

Threshold: AUTO Baseline Start Cycle: AUTO Baseline End Cycle: AUTO

Select a Target

Target	Threshold	Baseline Start	Baseline End
CMY-BID	15,000	3	10
CTX-M-14 BID	15,000	3	10
CTX-M-15 BID	15,000	3	10
IC BID	15,000	3	10

Ct Settings for CMY-BID

Ct Settings to Use Use Default Settings Automatic Threshold Automatic Baseline

Threshold: 15,000.0

Baseline Start Cycle: 3 End

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

Table 3. Optical channels (RUO) and threshold values determined during validation of the Streck ARM-D Kit, β -Lactamase on the ABI 7500 Fast 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	15,000	3	10
	CTX-M-15	HEX	HEX	15,000	3	10
	CTX-M-14	TEX615	Texas Red	15,000	3	10
	IC	TYE665	Cy5	15,000	3	10
PCR Mix 2	OXA-48	FAM	FAM	30,000	3	10
	IMP	HEX	HEX	30,000	3	10
	VIM	TEX615	Texas Red	30,000	3	10
	IC	TYE665	Cy5	30,000	3	10
PCR Mix 3	DHA	FAM	FAM	15,000	3	10
	KPC	HEX	HEX	15,000	3	10
	NDM	TEX615	Texas Red	15,000	3	10
	IC	TYE665	Cy5	15,000	3	10

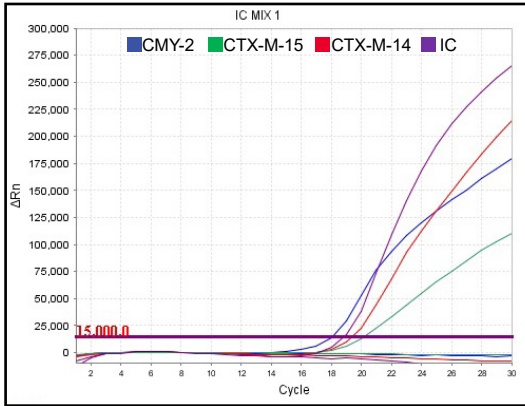
Amplification Curve Data

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

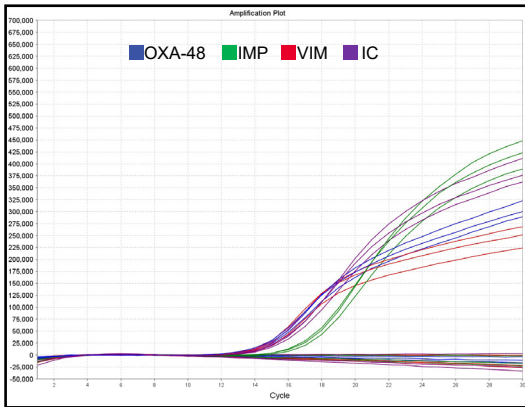
Characteristic amplification data for positive control targets detected with Streck ARM-D Kit, β -Lactamase (RUO) is shown in Figure 3. Although Cq values for amplification plot of unknown samples may vary from sample to sample, representative amplification data of β -lactamase-positive clinical isolates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

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

Control Mix 1



Control Mix 2



Control Mix 3

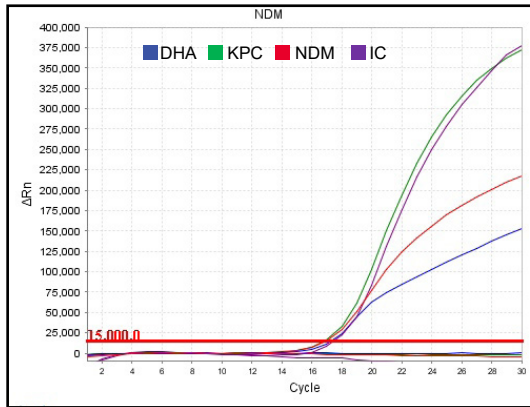
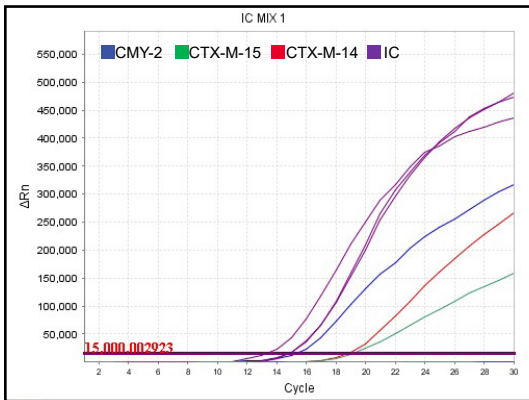


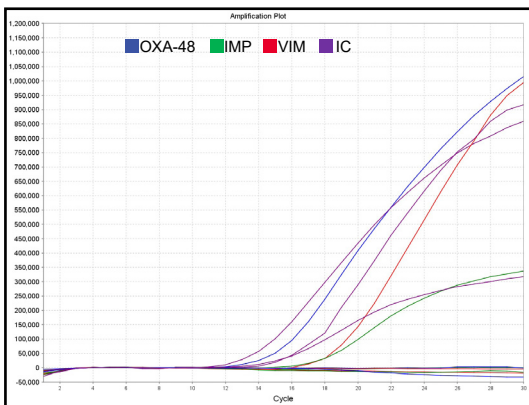
Figure 3. Multiplex real-time PCR amplification data of positive DNA Control Mixes of Streck ARM-D Kit, β -Lactamase (RUO) on the ABI 7500 Fast Real-Time PCR System.

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

PCR Mix 1



PCR Mix 2



PCR Mix 3

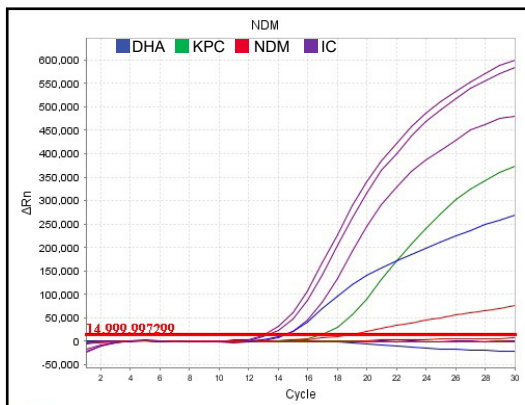


Figure 4. Amplification of β -lactamase-positive clinical isolates using Streck ARM-D Kit, β -Lactamase (RUO). Data shows the amplification of nine DNA samples that are positive for one of the respective β -Lactamase targets detected with the kit on the ABI 7500 Fast Real-Time PCR System. The IC (purple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

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)	16 ± 3
	CTX-M-15 (HEX)	18 ± 3
	CTX-M-14 (TEX615)	17 ± 3
	IC (TYE665)	17 ± 3
Control Mix 2	OXA-48 (FAM)	16 ± 3
	IMP (HEX)	17 ± 3
	VIM (TEX615)	16 ± 3
	IC (TYE665)	16 ± 3
Control Mix 3	DHA (FAM)	15 ± 3
	KPC (HEX)	15 ± 3
	NDM (TEX615)	15 ± 3
	IC (TYE665)	15 ± 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.

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.

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.

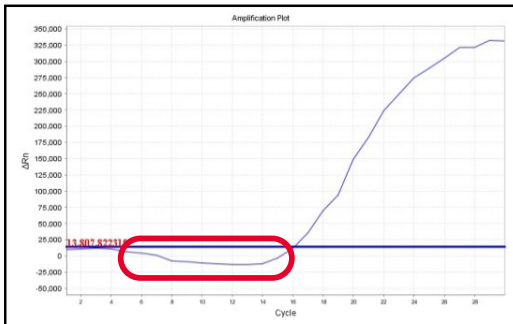
Negative Sample: If no Cq value is detected in the FAM, HEX, and Texas Red channels for unknown samples, confirm sample was added to the reactions by verifying positive amplification of the internal control (IC) for Gram-negative bacteria in the Cy5 channel (Cq = 10 - 20). If IC (Cy5) is amplified and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

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 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.

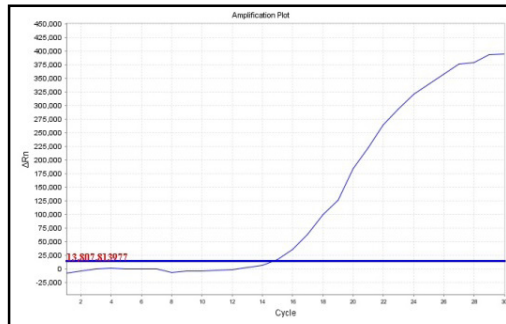
Troubleshooting

- 1. Extension time error message:** If Filter-3 (TAMRA, NED, Cy3 channel) is not de-selected during protocol setup as described above; an error message will appear in the screen prior to running the PCR protocol. The error message will indicate that holding time in the last cycling step must be over 20 seconds. No error message will be displayed if Filter-3 is deselected.
- 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 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.
- 3. Thresholding Algorithm Fail (THOLDFAIL) and Exponential Algorithm Fail (EXPFAIL) Flag on QC Summary occurs:** It is not uncommon that after data processing by the ABI 7500 Fast Real-Time PCR software, some samples will display a THOLDFAIL and/or EXPFAIL Flag in the QC Summary window. As described by the instrument manufacturer, the error message indicates failure to identify the threshold and 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. Usually these error messages will go away if the baseline/threshold values described in Tables 1 and 3 are manually set for each target combination.
- 4. 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:

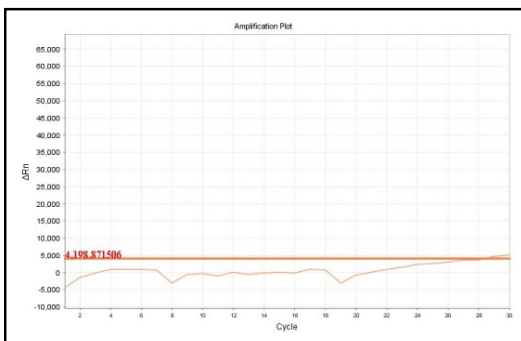
Incorrect baseline



Correct baseline



- 5. 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 (set at 6,500) 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.