

## Streck ARM-D<sup>®</sup> Kits

### Data Acquisition and Analysis Guide

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

#### Quick Links

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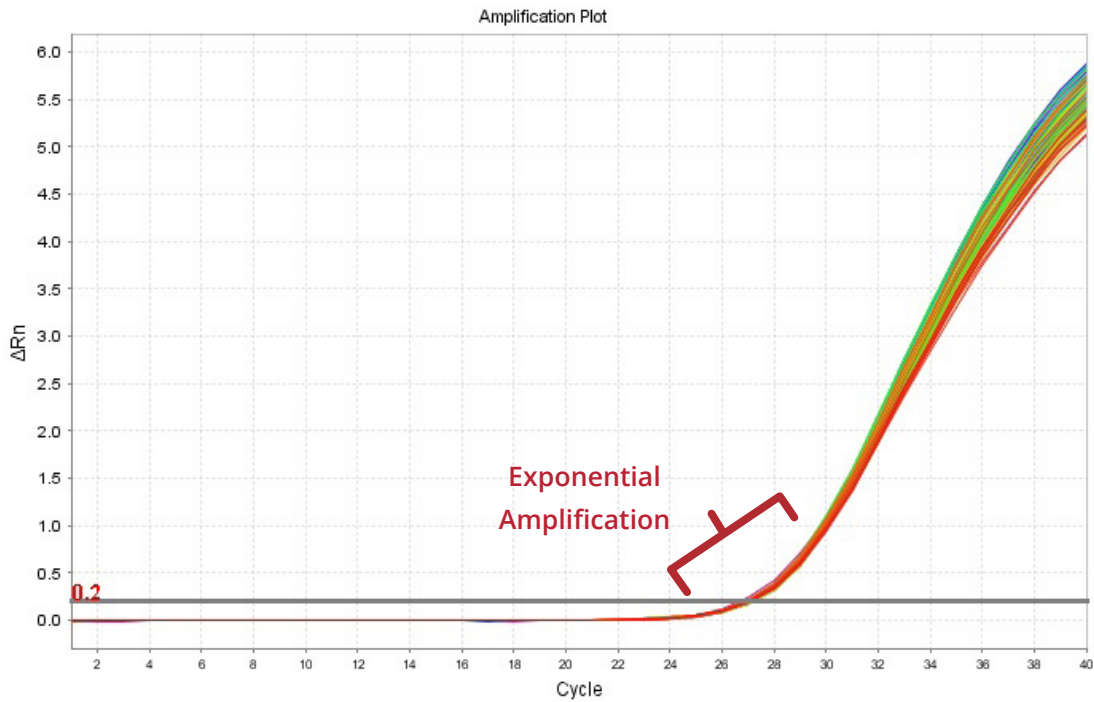
This guide is intended to be used as an ARM-D Kit-specific supplement for the Instructions For Use (IFU) document included with each kit. The Streck ARM-D kits referenced in this document are labeled as CE IVD and are For Export Only. Not for sale in the U.S. The instructions provided in this guide serve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

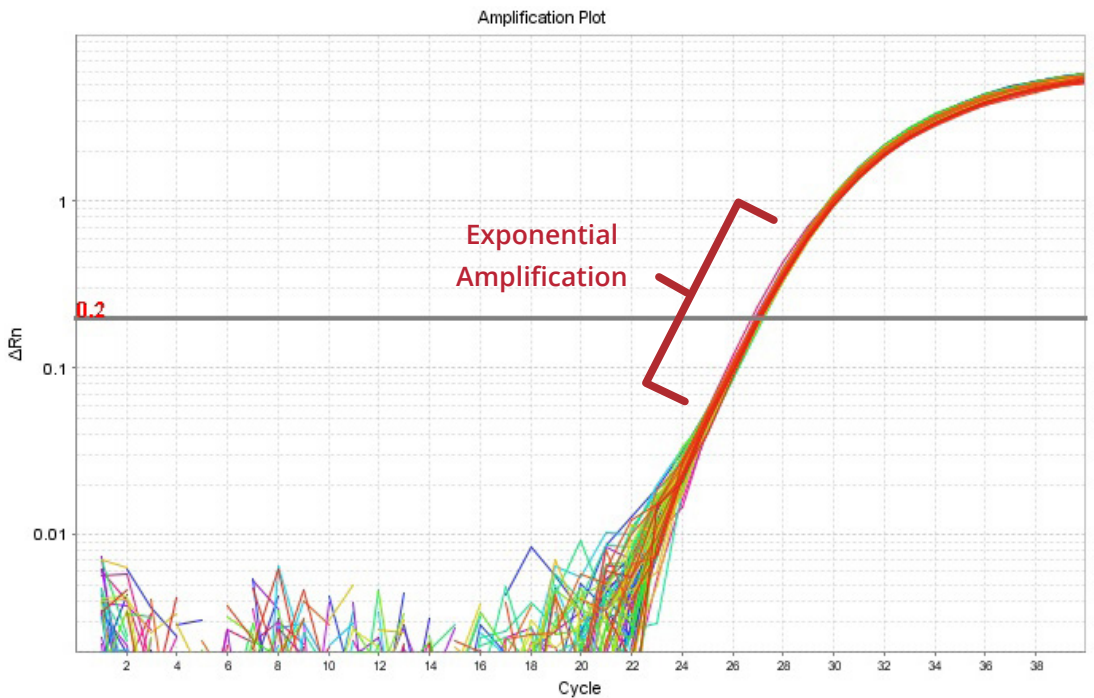
**Instrument and Protocol Set-up:** A template protocol can be made and reused for future assays to reduce instrument set-up time after the first run with each kit.

**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 (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).

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

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

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.

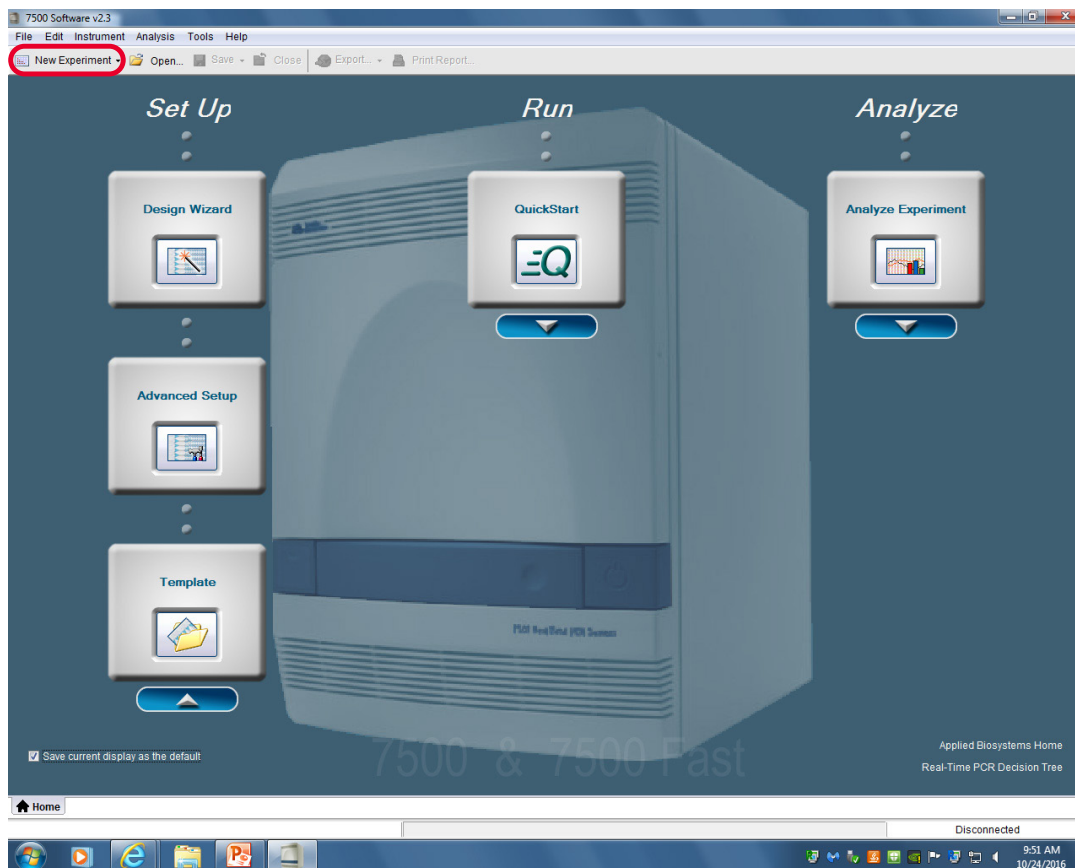
**Assay Performance:** It is expected that Cq values for positive controls and unknown samples should demonstrate amplification between cycles 10 and 26. Cq values determined for positive controls during internal validation are provided in Tables 2 and 4 for each respective kit as a guideline. Due to 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* and Streck ARM-D Kit,  $\beta$ -Lactamase.

### Instrument Set-up

Open the ABI 7500 Software.

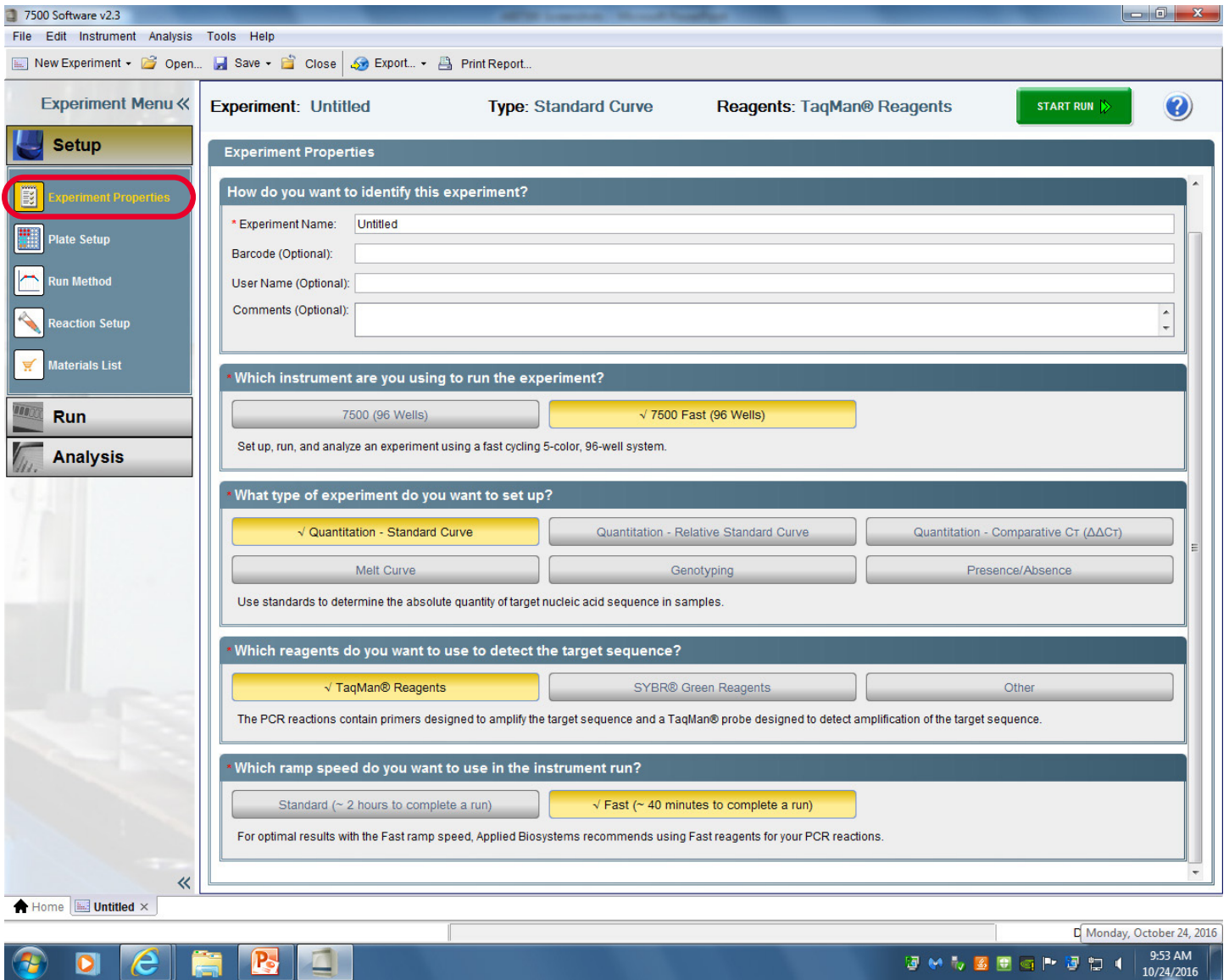
Click on New Experiment.



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



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

# 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

Define Targets and Samples Assign Targets and Samples

Instructions: Define the targets to quantify and the samples to test in the reaction plate.

**Define Targets**

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

**Define Samples**

Sample Name	Color
Control	Blue
NTC	Green

**Define Biological Replicate Groups**

Instructions: For each biological replicate group in the reaction plate, click **Add Biological Group**, then define the biological group.

Biological Group Name	Color	Comments
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Assign Targets and Samples

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.

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.

# 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

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	U S N	
<input checked="" type="checkbox"/>	ACC	U S N	
<input checked="" type="checkbox"/>	FOX	U S N	

Mixed U Unknown S Standard N 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		U FOX U ic		U FOX U ic								
B		U FOX U ic		U FOX U ic								
C		U FOX U ic		U FOX U ic								
D												
E												
F												
G												
H												

Wells: U 6 Unknown S 0 Standard N 0 Negative Control 90 Empty

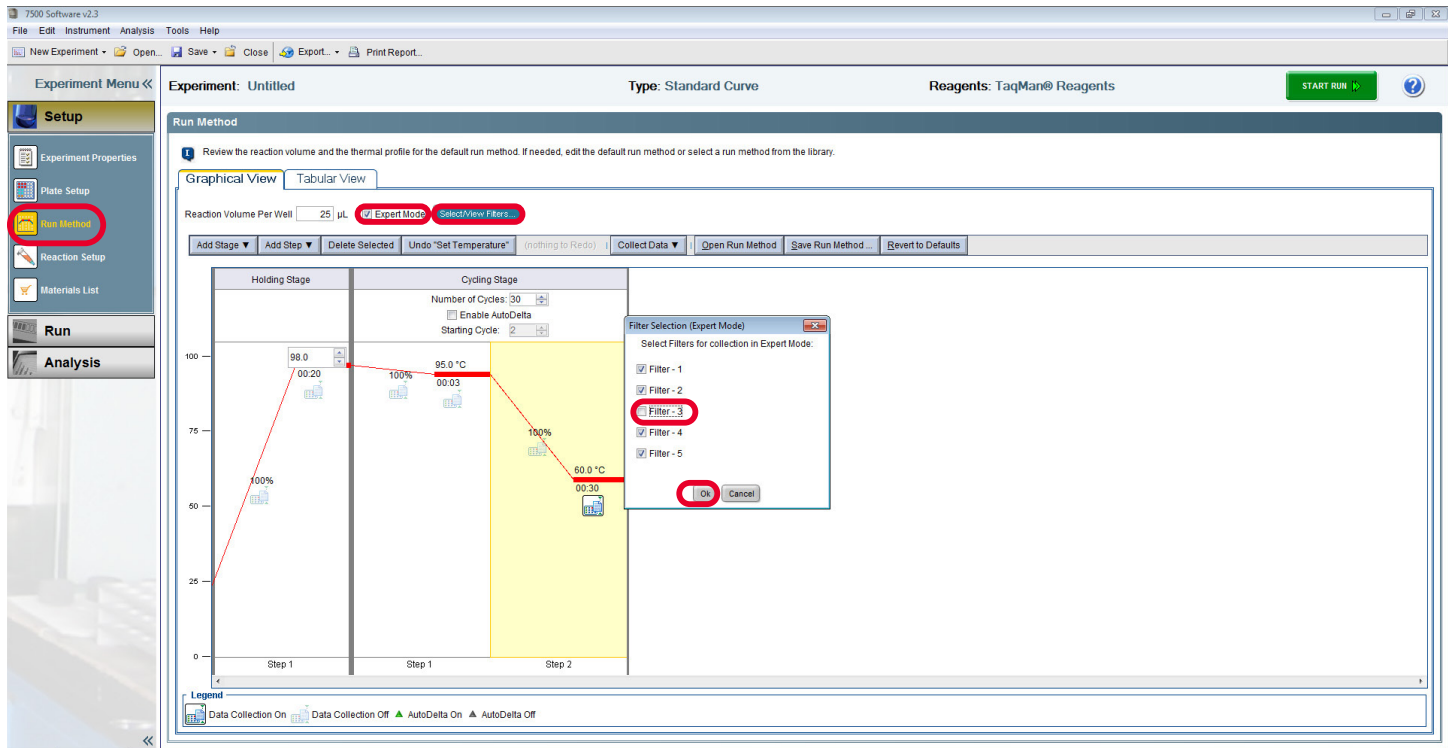
Home Untitled x

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.

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



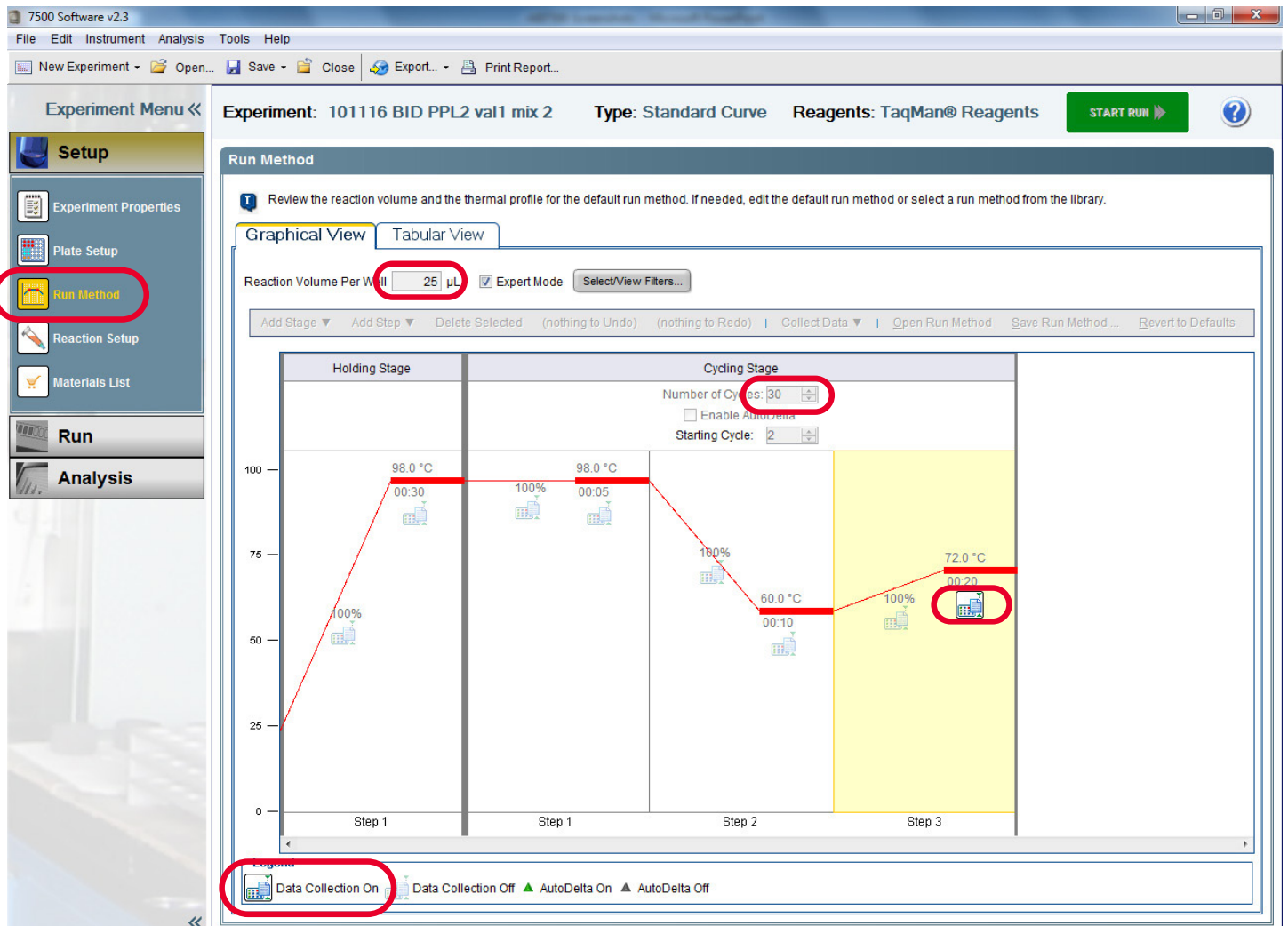
Enter the Streck ARM-D Kit protocol as shown below. Note that the PCR cycling protocol is the same for both Streck ARM-D *ampC* and  $\beta$ -Lactamase Kits.

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

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

- Change Reaction Volume to 25 µL.
- Change Number of Cycles to 30.
- Make sure Data Collection On is active after the extension step.

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



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.

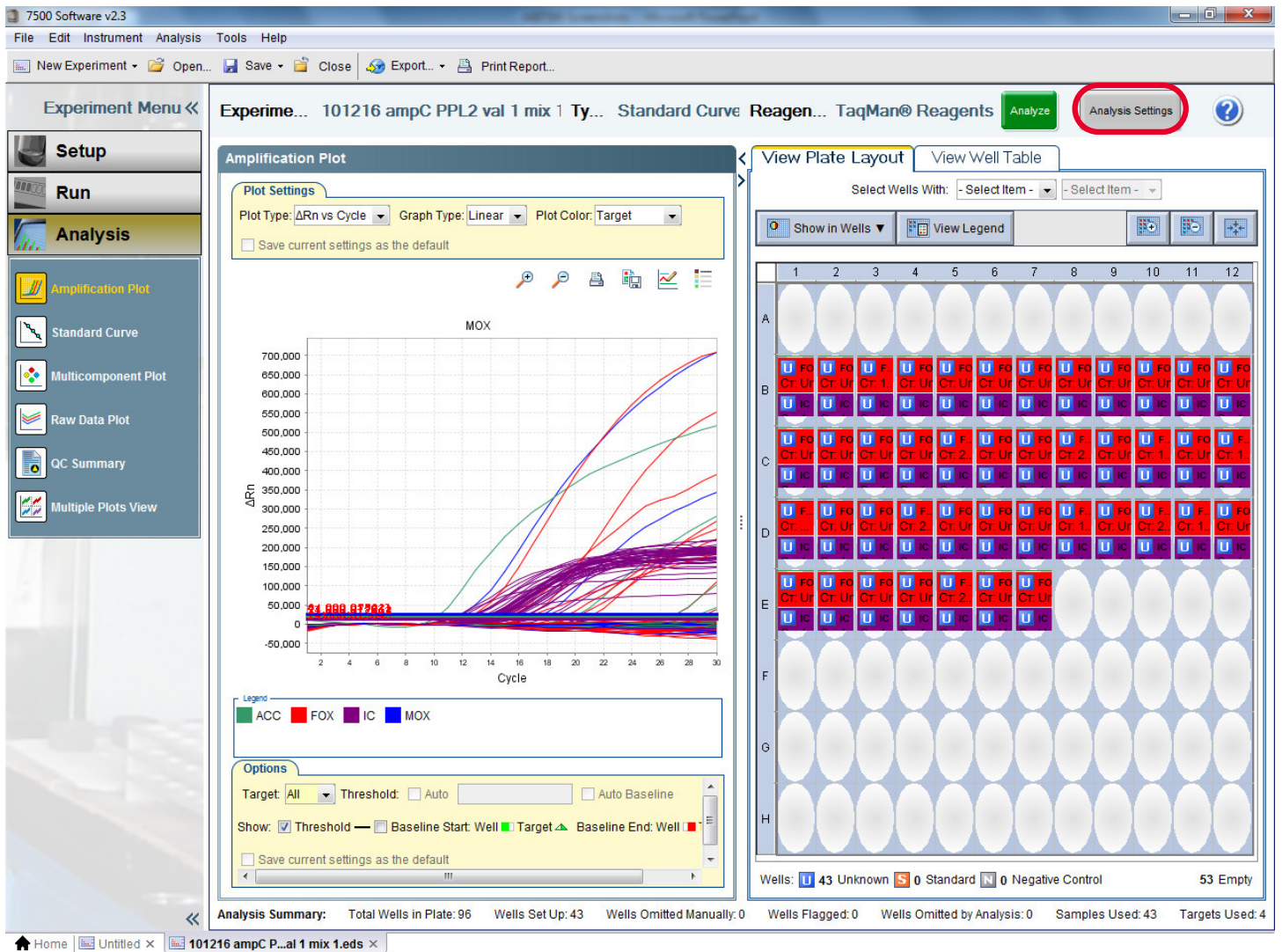
#### Threshold values and baseline settings

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.

\* 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

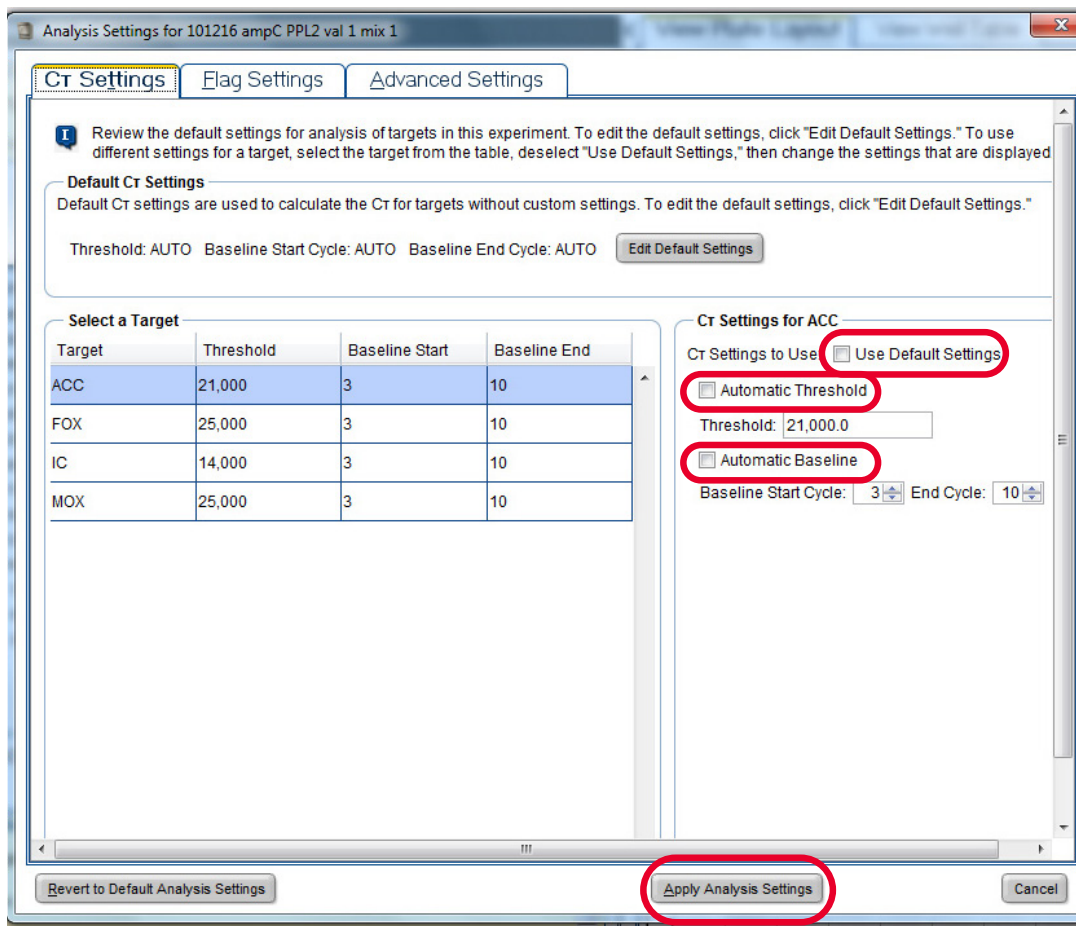


On the Ct Settings tab in the Analysis Settings window, deselect the following: Use Default Settings; Automatic Threshold and Automatic Baseline.

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/flourophore combination.

Click Apply Analysis Settings button.

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



**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

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

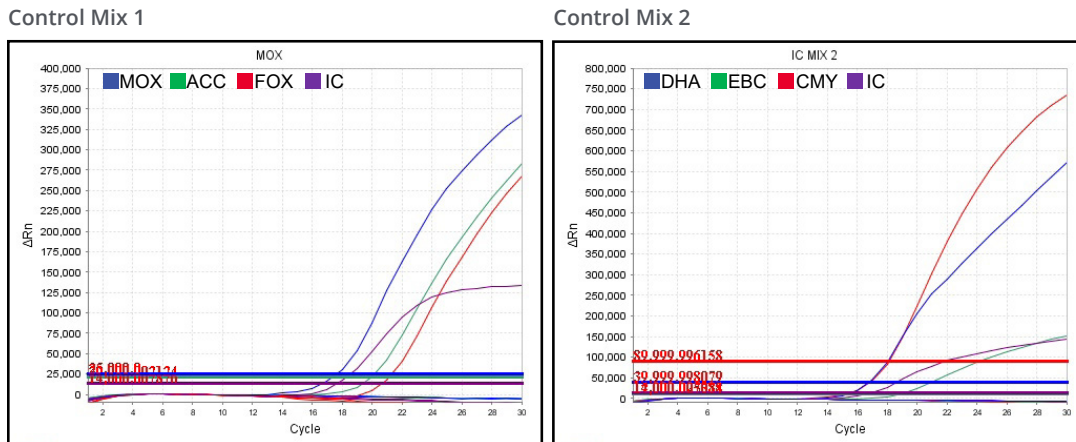


Figure 1: Multiplex real-time PCR amplification data of positive DNA Control Mixes for the Streck ARM-D Kit, ampC, on the ABI 7500 Fast Real-Time PCR System.

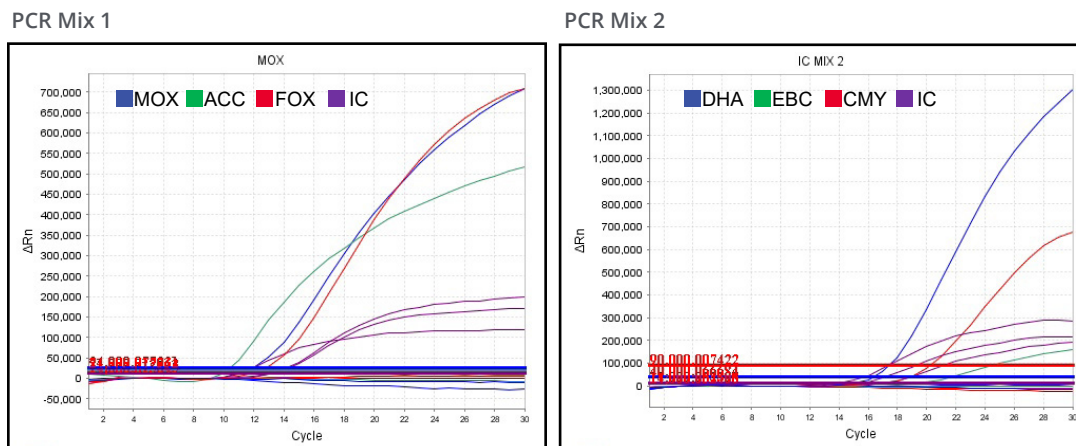


Figure 2: Amplification of plasmid-mediated ampC-positive clinical isolates using Streck ARM-D Kit, ampC. The data above shows amplification of six clinical isolates that are positive for each respective ampC target detected by the kit. The IC (purple lines) was detected in each sample.

## Cq Values – Controls

When setting threshold 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

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

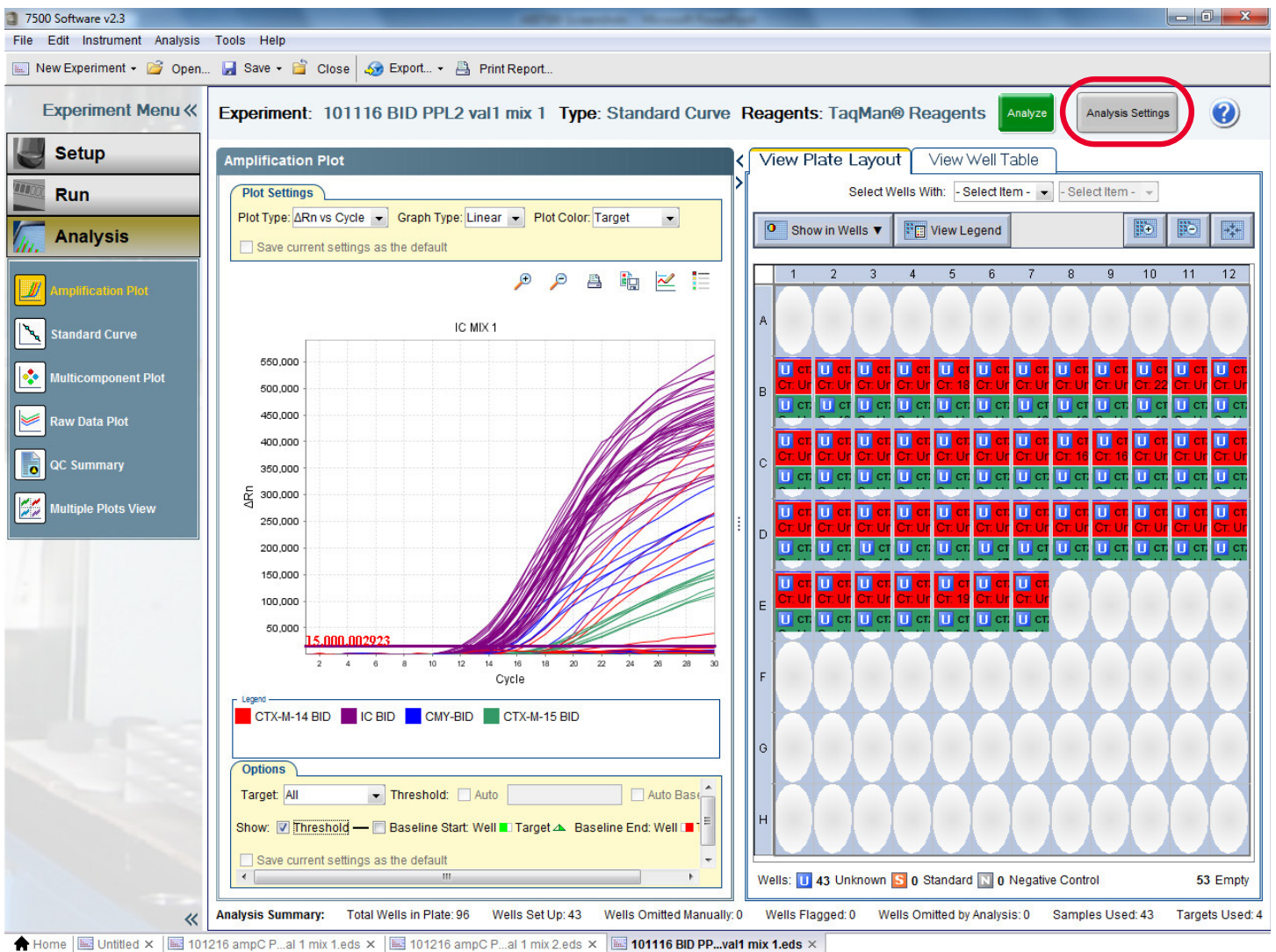
## Data Analysis and Data Interpretation: Streck ARM-D Kit, $\beta$ -Lactamase (RUO)

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.

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



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

## Streck ARM-D® Kits: Applied Biosystems (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

**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  
Threshold: 15,000.0  
 Automatic Baseline  
Baseline Start Cycle: 3 End

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

**Table 3:** Optical channels and threshold values determined during validation of the Streck ARM-D Kit,  $\beta$ -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

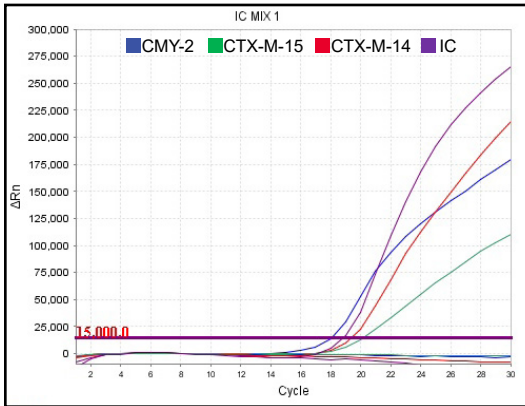
### Amplification Curve Data

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

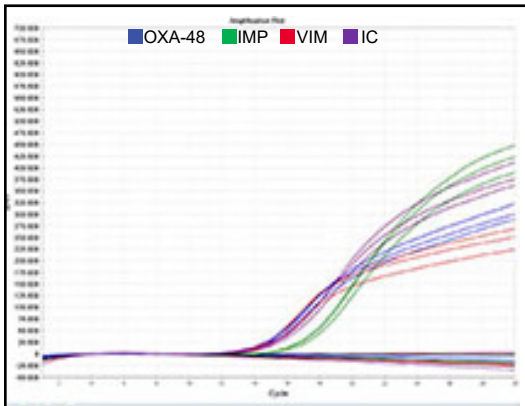
Characteristic amplification data for positive control targets detected with Streck ARM-D Kit,  $\beta$ -Lactamase is shown in Figure 3. Although C<sub>q</sub> values for amplification plot of unknown samples may vary from sample to sample, representative amplification data of  $\beta$ -lactamase-positive 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: Applied Biosystems (ABI) 7500 Fast Real-Time PCR System

Control Mix 1



Control Mix 2



Control Mix 3

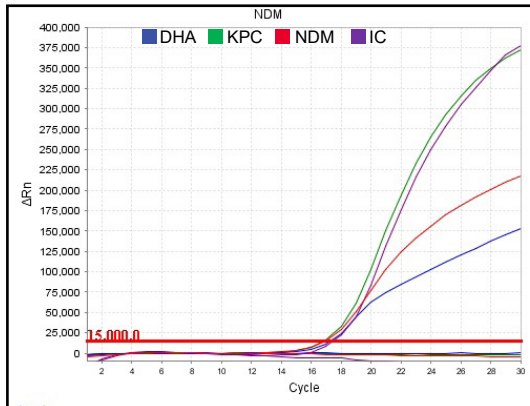
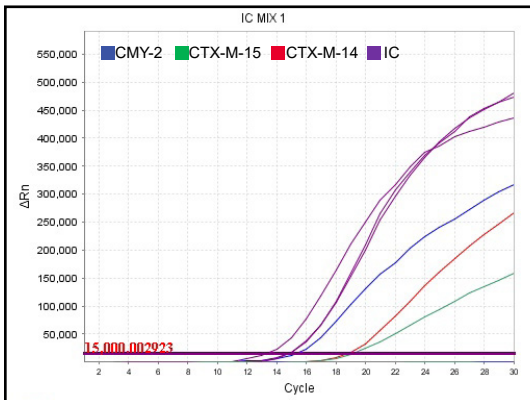


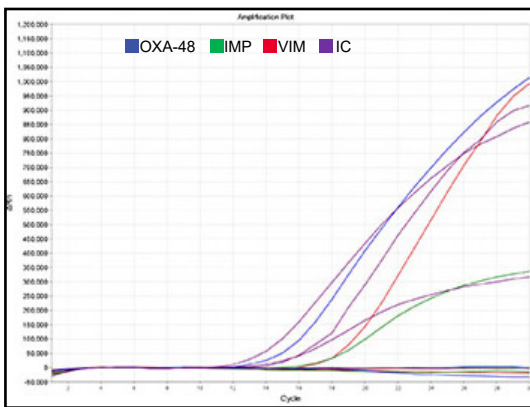
Figure 3: Multiplex real-time PCR amplification data of positive DNA Control Mixes of Streck ARM-D Kit,  $\beta$ -Lactamase on the ABI 7500 Fast Real-Time PCR System.

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

## PCR Mix 1



## PCR Mix 2



## PCR Mix 3

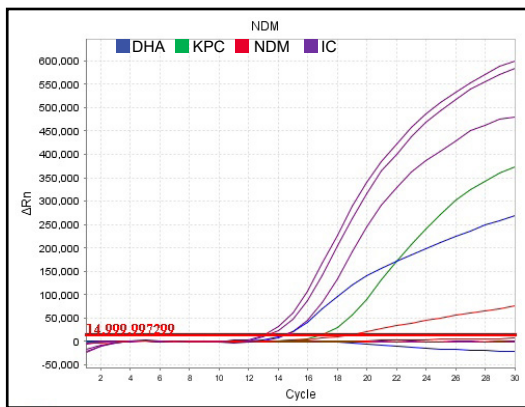


Figure 4: Amplification of  $\beta$ -lactamase-positive clinical isolates using Streck ARM-D Kit,  $\beta$ -Lactamase. Data shows the amplification of nine DNA samples that are positive for one of the respective  $\beta$ -Lactamase targets detected with the kit on the ABI 7500 Fast Real-Time PCR System. 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.

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.

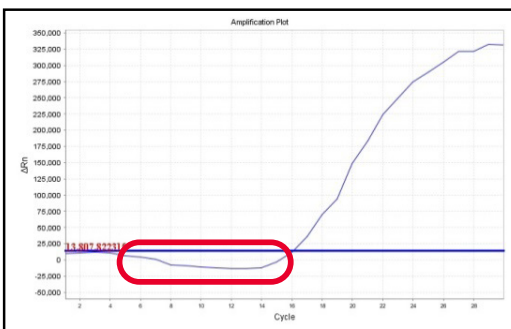
**Negative Sample:** If no Cq value is detected in the FAM, HEX, and Texas Red channels for unknown samples, confirm sample was added to the reactions by verifying positive amplification of the internal control (IC) for Gram-negative bacteria in the Cy5 channel (Cq = 10 - 20). If IC (Cy5) is amplified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

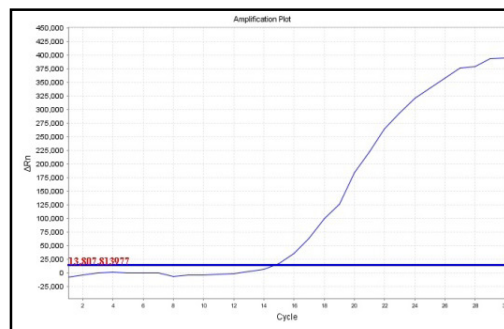
## Troubleshooting

- 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:

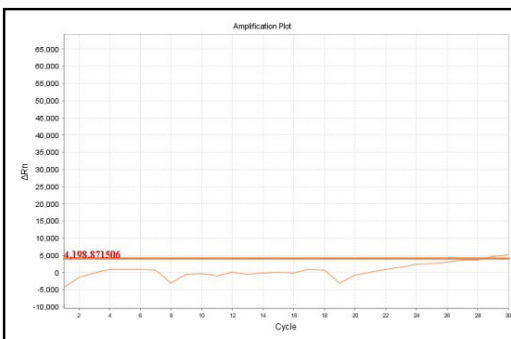
Incorrect baseline



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](mailto:technicalservices@streck.com).