

Frequently Asked Questions

Streck ARM-D[®] Kit, β -Lactamase and Streck ARM-D[®] Kit, *ampC*

Streck ARM-D Kits (RUO) are for Research Use Only. Not For Use in Diagnostic Procedures.
CE /IVD versions of these kits are also available. These kits are for Export Only. Not for sale in the U.S.

GENERAL INFORMATION

What targets does the Streck ARM-D Kit, β -Lactamase detect?

The β -Lactamase kit detects the following gene families; CMY-2, CTX-M-14, CTX-M-15, OXA-48, IMP, VIM, DHA, KPC and NDM. Each primer and probe set is designed to detect multiple variants in each family. A complete list of variants can be found on the Streck ARM-D Kit product page at streck.com.

What targets does the Streck ARM-D Kit, *ampC* detect?

The *ampC* kit detects MOX, DHA, ACC, ACT/MIR (EBC), FOX and CMY-2. Each primer and probe set is designed to detect multiple variants in each family. A complete list of variants detected can be found on the Streck ARM-D Kit product page at streck.com.

What are the Streck ARM-D Kits' clinical sensitivity and diagnostic specificity values?

The Streck ARM-D Kit, β -Lactamase has 99.9% sensitivity and 99.5% specificity based on the isolates tested during the product validation.

The Streck ARM-D Kit, *ampC* has 98.7% sensitivity and 99.5% specificity based on the isolates tested during the product validation.

What do the terms "clinical sensitivity" and "clinical specificity" mean?

The clinical sensitivity of a test is defined as the probability of correctly identifying a positive sample when the concentration of the analyte is at the lowest detectable concentration. It can be calculated as the ratio of correctly identified positive samples to known positive samples.

Clinical specificity or true negative rate corresponds to the probability of correctly identifying a negative sample. It can be calculated as the proportion of correctly identified negative samples in a population of known negative samples.

Are the same primers and probes used in the *ampC* and the β -Lactamase kits?

The β -Lactamase and *ampC* kits detect two targets in common: DHA and CMY-2. The sequences targeted are the same in both kits for the DHA-like genes. However, the CMY-2 sequences amplified by the two

kits are slightly different. Please refer to the target tables for each kit, which can be found on the Streck ARM-D Kit product page at streck.com. Please note that some variants identified by one kit in a sample, may not be identified by the other kit.

What is the method of detection used by the Streck ARM-D Kit assays?

This test is a hydrolysis probe, or TaqMan, assay. It includes a target sequence-specific primer pair and probe. The probe consists of conjugated fluorophore and quencher moieties so that the intact probe is not fluorescent. During primer extension, the probes are hydrolyzed by the 5' to 3' exonuclease activity of the DNA polymerase, causing the fluorescence emission to increase as the fluorophore is separated from the quencher. This fluorescence can be optimally detected at the end of the extension step of each PCR cycle, upon excitation with a light source of the appropriate wavelength.

What is the general process or workflow when using the Streck ARM-D Kits?

The Streck ARM-D Kit process includes the following steps:

- **Sample Collection:** A few (3 to 5) bacterial colonies are picked from an agar plate and deposited into a culture tube containing 5 mL of LB broth for overnight growth at 37 °C with shaking.
- **Sample Extraction:** 1.5 mL of the overnight culture containing approximately 10^{8-10} cells is extracted and purified using a manual extraction kit or an automated process.
- **Reaction Preparation:** Master mix preparation and reaction setup details are listed in each product's IFU.
- **Instrument Set-up/PCR Protocol:** This includes entering the PCR Protocol and creating the plate layout, followed by PCR amplification.
- **Data Interpretation:** Evaluation of each run will include manually setting the thresholds and evaluating each Cq value to determine the test results.

The IFU and Data Acquisition and Analysis Guides include details for each step on a particular instrument.

Frequently Asked Questions Streck ARM-D® Kit, β-Lactamase and Streck ARM-D® Kit, *ampC*

How long will it take to run a test with a Streck ARM-D Kit?

The overall time will depend on the methods used for each step. Sample collection will take approximately 12-24 hours for overnight growth. DNA extraction can take up to two hours. Reaction preparation can take up to 30 minutes. Run time could take up to one hour depending on the real-time cycler used.

What is the internal control (IC) target that is present in all mixes?

The internal control (IC) used in both Streck ARM-D Kits is an amplification control. The IC primers and probe amplify a region of the 16S rRNA gene that is common to over 95% of eubacteria. It verifies that the reaction proceeded correctly and that Gram-negative bacterial DNA was present in the sample. If a sample-containing reaction doesn't show amplification of the IC, meaning no Cq in the TYE665 channel (Cy5), the results from that well or tube should be carefully evaluated.

What is in the Control Mix vials?

The Control Mix vials contain DNA templates with the same sequences as those targeted by the primers and probes in the respective 10X PCR Mixes. They are used to verify that the reaction conditions (thermal set-up, enzyme activity, buffer composition, etc.) are appropriate for the targets being detected. When using 1 µL of a Control Mix in a 25 µL reaction containing 2.5 µL of the corresponding 10X PCR Mix, the expected Cq values for each target should match approximately the Cq values indicated in the Data Acquisition and Analysis Guide for the specific instrument.

How should the Streck ARM-D Kits be stored?

The Streck ARM-D Kits should be stored at -20 °C or below. The kit can be subjected to up to 15 freeze/thaw cycles, or openings, over its shelf life.

What sample type is needed for use with the kit?

The Streck ARM-D Kits were validated with extracted and purified DNA from isolated Gram-negative bacterial colonies, propagated in an overnight culture, as the sample type required for use with these kits. DNA purified from other starting sample sources may be used in a PCR. Please contact Streck Technical Services for recommendations.

Are the Streck ARM-D Kits capable of direct PCR?

At this time the kits have not been validated for direct PCR (amplification of samples without nucleic acid extraction, such as boil preps, or amplification of samples where DNA was purified directly from blood culture media, swab transport media, etc.). The sample type to include in the reaction is purified DNA from cultured bacterial colonies as specified by the IFU.

What specimen preparation methods may be used prior to using a Streck ARM-D Kit?

Streck has tested the kits using DNA extracted from bacterial isolates

using the QIAGEN DNeasy Blood and Tissue Kit. Various automated alkaline lysis techniques have also been used successfully. Ideally, the template should have a concentration >10 to 200 ng/µL and have an O.D. 260/280 ratio of 1.4 -2.4.

Can boil preps be used for sample extraction?

Streck has had success using the ARM-D kits on samples purified using a boil prep method. However, the concentration and purity should be as noted above.

REACTION PREPARATION

What other items are necessary to complete the testing procedure, but not included with the Streck ARM-D Kits?

The following reagents and equipment are necessary to successfully perform the testing procedure:

A qPCR-compatible DNA polymerase that has 5' → 3' exonuclease activity (TaqMan-compatible).

- Micro centrifuge.
- Vortex mixer.
- Pipettes and aerosol resistant tips of various volume ranges, from 1.0-1000 µL.
- A real-time PCR thermal cycler capable of exciting the kit probes and detecting their emission spectra.
- Optically clear PCR plates with seals, strips or tubes.

What types of tubes or plates are recommended for use with the ARM-D kits?

It is recommended that the tubes, plates, caps or adhesive covers be optically clear. Please consult the specific instrument user guide for the proper consumables. Several options, according to validated instruments, are listed below. These are not the extent of consumables that can be used on each instrument in conjunction with the Streck ARM-D kits.

Bio-Rad CFX-96

Vendor:	BioRad
Plates:	Hard Shell PCR Plates, 96 well, thin wall Part # HSP9641
Seals:	Microseal 'B' seal Seals Part # MSB1001
Tube strips:	0.2 ml 8-Tube PCR strips without Caps, low profile, clear Part # TLS0801
Tube strip caps:	0.2 ml Flat PCR Tube 8-Cap Strips, optical, ultraclear Part # TCS-0803

Frequently Asked Questions Streck ARM-D® Kit, β-Lactamase and Streck ARM-D® Kit, *ampC*

ABi QS7 and 7500 Fast Dx

Vendor:	Thermo Fischer
Plates:	MicroAmp EnduraPlate Optical 96-Well Fast Clear Reaction Plates with Barcode Part # 4483494
Seals:	MicroAmp Optical Adhesive Film Part # 4311971
Tube strips:	MicroAmp Fast Reaction Tubes (8 tubes/strip), 0.1 mL Part # 4358293
Tube strip caps:	MicroAmp Optical 8-cap Strip Part # 4323032

ABi 7500 Fast

Vendor:	MidSci
Plates:	0.1ml, MidSci #AVT3890
Seals:	Avant ThermalSeal optical plate seals, MidSci #TS-RT2-100

QIAGEN Rotor-Gene

Vendor:	QIAGEN
Tube strips:	QIAGEN 0.2 ml tubes, #981005
Tube strip caps:	QIAGEN 0.1 ml strip tubes and caps, #981103

What type of pipettes and tips should be used with the Streck ARM-D Kits?

A variety of pipettes and tips are needed to properly prepare the master mix, transfer it into individual PCR reaction tubes or wells and add template DNA. The pipette dispense ranges should include; 1-10 µL, 5-20 µL, 20-200 µL and 100-1000 µL. Streck recommends using low bind, PCR clean, aerosol-resistant pipette tips.

Is there an alternate polymerase to the Supermix 2X?

Hot start polymerases compatible with TaqMan probes may work for this assay; reaction reagent volumes, reaction temperatures, and hold times during thermal cycling will need to be adjusted to account for different reaction chemistries. Other polymerases will potentially result in different threshold and baseline settings for Cq determinations, as well as different target sensitivities and specificities.

How many control samples are necessary for the procedure?

At a minimum, one positive and one negative template control (NTC) are needed to ensure the fidelity of the test.

How many NTC (no template) reactions are required for the reaction preparation?

It is recommended to use a minimum of one NTC for each PCR mix. It is good practice to prepare an NTC reaction at the time the Master Mixes are prepared, and a second one at the time the rest of the samples are added to the plate or tubes, which can help differentiate

contaminated reagents from contamination at the lab bench during unknown sample addition.

How long should each vial be mixed on the vortex and centrifuged prior to opening?

Typically, 5 seconds is sufficient for each vortex and centrifugation step.

What is the best method for mixing the master mix?

Consistent results have been achieved by mixing with the pipette or by using a vortex mixer followed by brief centrifugation.

How long should the PCR plate or tubes be centrifuged prior to running on the instrument?

The purpose of this step is to ensure that all reaction components are localized at the bottom of the PCR tube or well.

Typically 30 seconds of centrifugation in a microfuge tube or 2 minutes at 250xg will suffice for centrifugation of a PCR plate. Inspect the PCR plates or tubes prior to placing the samples in the thermal cycler to ensure that no liquid is present on the plate film or tube cap.

INSTRUMENT SET-UP/PCR PROTOCOL

What thermal profile should be used with the Streck ARM-D Kits?

The recommended PCR protocols are:

Stage	General Protocol	ABi 7500 Fast Dx
Hot Start	98 °C for 30 sec	98 °C for 30 sec
30 cycles of:	98 °C for 5 sec	98 °C for 10 sec
	60 °C for 10 sec	60 °C for 15 sec
	72 °C for 20 sec	72 °C for 30 sec
	(Detection Step)	(Detection Step)

Streck has validated the amplification protocol in the following instruments: Applied Biosystems QuantStudio 7 (QS7) Flex Real-Time System, Applied Biosystems 7500 Fast and 7500 Fast Dx Real-Time PCR Systems, Bio-Rad CFX96 Touch Real-Time PCR Detection System, QIAGEN Rotor-Gene Q, and Streck Zulu RT® PCR System. The performance specifications of a different instrument used for amplification of Streck ARM-D Kits may affect the results, and therefore, the PCR protocol may need to be optimized if using other qPCR platforms.

On what instruments can the Streck ARM-D Kits be used?

The Streck ARM-D Kits have been validated on the Applied Biosystems QuantStudio 7 (QS7) Flex Real-Time System, Applied Biosystems 7500 Fast and 7500 Fast Dx Real-Time PCR System, Bio-Rad CFX96 Touch Real-Time PCR Detection System, QIAGEN Rotor-Gene Q, and Streck Zulu RT PCR System. Other 4-color instruments

Frequently Asked Questions Streck ARM-D® Kit, β -Lactamase and Streck ARM-D® Kit, *ampC*

with the ability to detect the following channels may be suitable for use with these kits:

Dye	Excitation max	Emission max	Compatible channels (example)
FAM	495 nm	520 nm	FAM
HEX	538 nm	555 nm	HEX/VIC/JOE
TEX615	596 nm	613 nm	Texas Red/ROX
TYE665	645 nm	665 nm	Cy5

Can the Streck ARM-D Kits be used on a StepOne Real-Time PCR System?

The basic StepOne System has a 3-color optical system which is not sufficient for use with the Streck ARM-D Kits, which have four fluorescent probes. Upgraded systems, such as the StepOnePlus System, can detect four channels, but would likely need calibration to the four colors specific to the Streck ARM-D Kits.

Can the Streck ARM-D kits be used on the Roche Light Cycler 480 II?

For those wishing to utilize the Streck ARM-D kits on the Roche Light Cycler 480 II system, it is recommended that they contact Streck for information regarding color compensation for the specific fluorophores included in the kits.

What should be done if an error message occurs when entering the protocol hold times?

The PCR cycling protocol described in the Instructions For Use (IFU) includes the recommended temperatures, and times for each protocol step. The hold times listed are the shortest times recommended for optimized performance. These hold times may vary with different software and instrument versions. If an error message is displayed on the instrument's graphical user interface just before starting the PCR run, please consult the instrument manual; also, please verify that data are collected in no more than the four channels required by the kit. Any additional channels that remain activated may cause an increase in the time required to cycle. Some instruments may require a longer time for data acquisition during each cycle; if this is the case you may use the minimum time required by your instrument. Specific instructions to setup PCR protocols for select instruments are included in the Data Acquisition and Analysis Guides.

What quencher should be set in the analysis software?

You do not have to set a quencher for this assay if this option is requested by the software. If it is mandatory to enter a quencher, select None in the analysis parameters.

What can be done if the real-time PCR instrument does not have a calibration for the fluorophores used in a Streck ARM-D Kit?

If the instrument is not calibrated for the fluorophores necessary for use with the Streck ARM-D Kits, use a compatible detection channel as an alternative or calibrate the instrument for the specific protocol. It should be noted that Cq values may change slightly when a fluorophore is not calibrated but read on a compatible channel. The excitation and emission maxima of the fluorophores in the Streck ARM-D Kits are:

Dye	Excitation max	Emission max	Compatible channels (example)
FAM	495 nm	520 nm	FAM
HEX	538 nm	555 nm	HEX/VIC/JOE
TEX615	596 nm	613 nm	Texas Red/ROX
TYE665	645 nm	665 nm	Cy5

Will adding an additional 5-10 seconds to the extension/detection step affect the Cq values?

Typically, a 5-10 second increase to the extension time will not affect Cq values.

DATA INTERPRETATION

What threshold values should be used with the Streck ARM-D Kits?

Streck recommends manually setting the threshold values for each target detected by the kits. Instrument-specific threshold settings for each of the instruments validated for the Streck ARM-D kits are listed in the Data Acquisition and Analysis Guides. If the instrument is not listed, please contact Streck Technical Services. When setting threshold values, they should be set within the linear phase of exponential amplification for all samples under analysis. It should be high enough to avoid background noise, but low enough that the reactions being analyzed reach that value during their exponential phases of amplification. The threshold for each target should be adjusted for optimal placement to achieve the correct Cq. The threshold values will be different when using different analysis platforms.

What cycle range should be used to set the baseline when analyzing data generated from a Streck ARM-D Kit?

Each analyzer may use a differing range of cycles to determine the baseline correction. Consult the instrument manual and the Streck ARM-D Kit Data Acquisition and Analysis Guide for a specific instrument.

How will the results be affected if the incorrect amount of template is added?

If too little sample is added, the Cq for a positive sample may appear

Frequently Asked Questions Streck ARM-D® Kit, β -Lactamase and Streck ARM-D® Kit, *ampC*

after the suggested 10-26 cycle range for a positive result. If too much sample is added the resulting Cq values will be too low for proper analysis. Some software platforms will ignore Cq values less than 10 while others will incorrectly assign baseline cycles, resulting in erroneous results.

What is the significance of a Cq value outside of the recommended range?

In general, Cq values higher than 26 cycles are considered a negative or undetermined result. However, each sample should be carefully examined as several factors could affect the reaction such as the presence of PCR inhibitors or low template DNA concentration. In the case of high Cq, verify the sample's DNA concentration and O.D. 260/280 ratio for purity, and re-extract the sample if necessary. A Cq reading under 10 cycles usually indicates an overloading of the template DNA, but it may be the result of a software error such as an incorrect baseline setting or a threshold value set incorrectly. If correct baseline and thresholds settings are applied and a Cq reading is above 10 cycles, less template volume or diluted template may be used to repeat the reaction and shift the Cq into the 10 to 26 cycle range.

What can be the cause of Control Mixes that result in no Cq value?

Possible reasons include:

- Control Mix was not added to the PCR well/tube.
- A Control Mix was added to a reaction with the incorrect 10X PCR Mix (e.g., Control Mix 1 was added to 10X PCR Mix 2 reaction). In this case, only the IC should have resulted in amplification.
- The DNA polymerase or other PCR reagent was not added to the reaction mix. This would likely affect all samples that used the same master mix.
- The reagents have degraded through excess freeze/thaw cycles or are expired.
- Incorrect parameters for data acquisition and analysis may have been applied to the PCR run (ex., ROX normalization was selected).

What could be the reason for not recovering identical results for duplicate tests on the same sample?

Cq values may vary somewhat between individual reactions. Variations of more than 1 cycle in the Cq values of 2 technical replicates may indicate:

- Pipetting errors when dispensing template or master mix to the wells.
- Contamination of a well with exogenous template or PCR inhibitors.
- Variations in thermal properties in the block.
- Erroneous baseline range determination.

Why is the internal control target not amplifying (no internal control Cq)?

Some reasons may include:

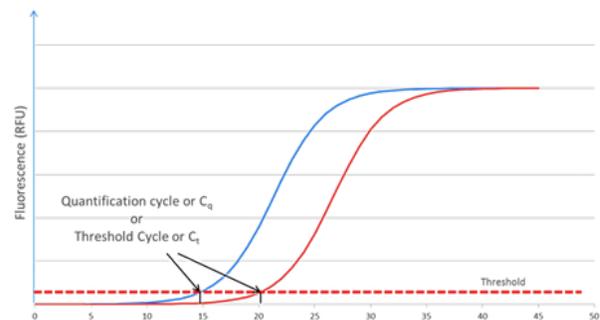
- DNA sample was not added to the PCR well/tube.
- Failed DNA extraction; verify/check the DNA concentration and O.D. 260/280 ratio and re-extract if necessary.
- The extracted DNA is not from a Gram-negative bacteria.

The bacterial species in the sample is among the 5% of Gram-negative bacteria that do not contain the region of the 16S rRNA gene targeted by the primers/probe.

What is the difference between Cq, Ct, and Cp?

These and other similar terms can be considered synonymous. They are used by different software platforms to describe the fractional cycle number at which a reaction should be quantified or compared to another sample where the same target is being detected by the same fluorescent probe.

Real-time PCR is based on the principle that the time necessary for an amplification reaction to reach an amount of template concentration is inversely proportional to the amount of template present in the sample prior to amplification. Through the use of fluorescent probes, the measured increase in fluorescence intensity with increasing cycles correlates with amplified target concentration. The fluorescence value that needs to be reached in order to quantify or compare samples is the threshold value.



Streck ARM-D Kit, β -Lactamase (RUO) and Streck ARM-D Kit, *ampC* (RUO) are for Research Use Only. Not for use in diagnostic procedures.

Streck ARM-D Kit, β -Lactamase and Streck ARM-D Kit, *ampC* are CE IVD - Export Only. Not for sale in the U.S.