Streck ARM-D® Kit, TEM/SHV/GES (RUO)

INSTRUCTIONS FOR USE

INTENDED USE

The Streck ARM-D® Kit, TEM/SHV/GES (RUO) is a qualitative molecular test for the detection of three ESBL gene families by fluorescently-labeled DNA probes. Positive identification of the gene by this test indicates the presence of these resistance genes. The Streck ARM-D Kit, TSG (RUO) generates data in under one hour. **This product has not been cleared by the U.S. Food and Drug Administration for In Vitro Diagnostic use. The product is For Research Use Only (RUO). Not for use in diagnostic procedures.**

INTRODUCTION

The dissemination on plasmids of the common extended-spectrum β-lactamases (ESBLs) TEM, SHV, and GES have been documented across Gram-negative bacteria in multiple countries for over the last two decades. Molecular detection is important for screening of these pathogens, infection control, epidemiologic data, and supplementing phenotypic testing. The Streck ARM-D kit, TSG (RUO) provides detection of TEM, SHV, and GES β-lactamase gene families by real-time PCR.

SUMMARY AND PRINCIPLES

Nucleic acid tests can identify specific resistance mechanisms associated with antibiotic resistance. As such, this information can be supplemented with susceptibility testing to support test results. Streck ARM-D Kit, TSG (RUO) allows for identification of three ESBL gene families: TEM, SHV and GES. Additionally, an endogenous internal control (IC) that targets a conserved region common in Gram-negative bacteria is included to reduce false negatives due to PCR inhibition, DNA degradation, or poor extraction. This test utilizes sequence-specific primer pairs for the PCR amplification of each family as well as fluorophore-labeled, target-specific DNA probes for detection by real-time PCR.

This product has been validated with the Applied Biosystems (ABI) 7500 Fast Real-time PCR System.

CONTENTS

The kit includes one multiplex mix vial, containing all required primers and probes, in TE buffer, pH 8.0 (10X PCR Mix) for simultaneous real-time PCR amplification of all targets. One control vial (Control Mix) containing synthetic DNA templates of the corresponding multiplex targets are also included in the kit to use as an external positive DNA control for the multiplex reaction. A premixed 2X Supermix vial containing buffer, dNTPs, MgCl2, and DNA polymerase are also included in each kit. The kit contents are enough for 100 reactions, including 12 reactions of the control mix.

Primer/Probe Vials	Control Vials	Cap Color	Target Genes
10X PCR Mix	Control Mix	Red	TEM, SHV, GES, IC

***IC is the Internal Control Gene, 16S rRNA.**

PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. Use established precautions with potentially biohazardous specimens according to your laboratory guidelines.
3. Always use DNase/RNase-free plasticware/reagents and aerosol-barrier pipet tips.
4. SDS can be obtained at streck.com, by calling 800-843-0912, or by calling your local supplier.

STORAGE AND STABILITY

1. When stored at -20 °C +/- 5 °C, unused kit contents are stable through the expiration date.
2. Minimize the number of freeze-thaw cycles where possible. Aliquots of the reagents for long-term storage may be prepared.
3. When using reagents for consecutive days, store at 4 °C. Store at -20 °C +/- 5 °C for extended storage periods.

SAMPLE EXTRACTION

The Streck ARM-D Kit, TSG (RUO) was validated with previously characterized DNA samples extracted from pure bacterial culture using the QIAGEN DNeasy Blood and Tissue Kit. 1.5ml of a 5ml overnight culture was used as per the extraction kit protocol yielding DNA concentrations that range from 10-200ng/µl, with 260/280 ratios that range from 1.4 to 2.4. Alternative growth protocols for pure bacterial cultures and nucleic acid extraction techniques/kit should also give DNA of enough yield and quality. The 30-cycle PCR assay has not been tested for use with clinical samples in which targets are present in low DNA copy numbers (e.g., direct, uncultured samples).

REACTION PREPARATION

Thaw reagents, vortex briefly to mix contents, and pulse-spin vials prior to opening. Prepare a master mix (without template DNA) according to the table below and based upon the number of samples to be processed (plus one extra reaction). Include at least one Control Mix reaction and two no-template-control (NTC) samples for each respective PCR run.

Mix well by pipetting up and down several times. Aliquot 24µl of master mix into each real-time PCR well or tube. Add 1µl of unknown sample, Control Mix, or nuclease-free water (for NTC) to the master mix within the respective PCR well or tube. It is recommended to run two NTC samples; one at PCR set-up to test for contaminated reagents and one after the addition of template to test for carryover during template distribution. Centrifuge PCR plate or tubes prior to loading into the respective instrument.

Source	Component	25µl Reaction	Final Concentration
Lab Supplied	Nuclease-Free Water	9.0µl	NA
Streck ARM-D Kit	Supermix 2X	12.5µl	1X
Streck ARM-D Kit	10X PCR Mix	2.5µl	1X
Distribute Master Mix into PCR wells or tubes as appropriate before sample addition			
Lab Supplied or Streck ARM-D Kit	Template - Unknown or NTC or Template - Control Mix	1µl	Variable

PCR PROTOCOL

The following protocols have been optimized for use with the supplied Supermix 2X master mix. Some instruments may require longer extension time for signal acquisition (Detection Step). Consult your instrument manual for additional information.

Step	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 60 °C for 10 sec 72 °C for 20 sec (Detection Step)	98 °C for 10 sec 60 °C for 15 sec 72 °C for 30 sec (Detection Step)

INSTRUMENT SET-UP

The detection of each target is based on the fluorescence of the fluorophore conjugated to each target-specific DNA probe as shown in the table below. The following are general instrument set- up instructions. Parameters specific to the ABI 7500 Fast Real-time PCR System are described in the Data Acquisition and Analysis Guide which can be found on streck.com.

1. Insert plates or tubes into the real-time PCR system.
2. Create or select a thermal profile or cycling protocol.
3. Assign control and sample wells when necessary.
4. For data interpretation, thresholds should be manually set for optimal performance (see Data Acquisition and Analysis Guide for recommended instrument-specific threshold and baseline settings).

Table 1. The detection of each target is based on the optical fluorescence of the fluorophore conjugated to each target-specific DNA probe.

Master Mix	Target Gene	Fluorophore	Excitation λ_{max}	Emission λ_{em}
PCR Mix	TEM	FAM	495nm	520nm
	SHV	HEX	538nm	555nm
	GES	TEX615	596nm	613nm
	IC	Cy5	645nm	665nm

DATA INTERPRETATION

General: Each real-time PCR run must be validated with the Control Mix vial provided with the kit. If the specifications for Cq values for the DNA control are not met, the results are considered invalid and samples must be re-evaluated. Cq values of unknown samples will vary depending on the starting DNA copy number. Visually inspect amplification curves for each unknown sample to verify results. As a general guideline, Cq values for TEM, SHV. and GES targets in unknown isolates can range from 10 to 26.

The Streck ARM-D Kit, TSG (RUO), is a qualitative test. To verify performance of the kit, each real-time PCR run must be verified with the control mix vial provided with the kit and by evaluating positive and negative control amplification curves.

1. Cq values for positive controls may vary between real-time PCR systems. For optimal assay performance, verify that threshold values for each target and/or fluorophore have been manually set prior to analyzing Cq values for unknown samples, (See instrument-specific Data Acquisition and Analysis Guide for more information).
2. Control samples will have a positive Cq value in the FAM, HEX, TEX615, and Cy5 channels. If the Cq value is ≤ 26 for each target, control runs should be considered valid.
3. Negative Controls should not have a Cq value.
4. If there is a run failure on the real-time PCR system, results are invalid, and the assay must be repeated.
5. Unknown samples may be interpreted as positive if the Cq value is ≤ 26 cycles.
6. Cq values of unknown samples will vary depending on the starting DNA concentration. If no Cq value is detected in the FAM, HEX, and TEX615 channels for unknown samples, confirm sample was added to the reactions by verifying positive amplification of the internal control (IC) in the Cy5 channel., which can be detected with the PCR mix included in the kit.
7. If no amplification is detected with the unknown sample, the sample may be interpreted as negative for the targeted resistance mechanisms.
8. If amplification of an unknown sample in the FAM, HEX, and TEX615 channels is detected after 26 cycles, the sample requires further investigation. The sample may be re-extracted, the PCR run repeated, or the amplified product could be sequenced for verification.
9. If Cq values for control targets or unknown samples fall outside the indicated range, please contact Streck Technical Services for further assistance at 800-843-0912 or technicalservices@streck.com.

Notes:

1. As a guideline for determining target- and instrument-specific Cq values for each control, please reference the instrument-specific Data Acquisition and Analysis Guides at streck.com. These values were determined during Streck's internal validation of the assay for each control target and real-time PCR system indicated.
2. In this IFU, the term Cq (Quantification Cycle) indicates the cycle number at which fluorescence from amplification exceeds the background fluorescence as per recommendation by MIQE Guidelines. However, depending on the real-time PCR system manufacturer, the term has also been referred to as threshold cycle (Ct) or crossing point (Cp).

LIMITATIONS

1. The internal control (IC) primers have been designed to amplify a highly conserved gene target present in many Gram-negative bacteria. However, the IC may not successfully amplify from certain Gram-negative species or strains. Therefore, one should consider this for interpreting the absence of the IC product from a specific sample.
2. The gene family targets have been tested against a considerable number of isolates with excellent sensitivity and specificity results. The PCR primers will only amplify the specified target families and will not detect other genes. Extensive testing has been done in DNA extracted from *Escherichia*, *Klebsiella*, *Salmonella*, and *Enterobacter* genera. However, given the genomic diversity of bacteria, Streck does not guarantee that all TEM, SHV, and GES genes will be detected in all Gram-negative subspecies. Results from this test should be used in combination with other laboratory tests available for accurate interpretation.
3. Using the Streck ARM-D Kit, TSG (RUO) with alternative 4-channel real-time PCR platforms or other enzymes not listed in this IFU is possible, but optimization may be required. Contact Streck Technical Services for assistance.

REFERENCES

1. Shah A.A., Hasan F., Ahmed S., Hameed A., 2001. Characteristics, epidemiology and clinical importance of emerging strains of Gram-negative bacilli producing extended-spectrum beta-lactamases. Res Microbiol. 155(6):409-21.
2. Naas T., Poirel L., Nordmann P., 2008. Minor extended-spectrum beta-lactamases. Clin Microbiol Infect. 14, Suppl 1: 42-52.
3. Jacoby G.A., Bush K., 2005. Beta-lactamase nomenclature J Clin Microbiol. 43(12):6220.

ORDERING INFORMATION

Please call our Customer Service Department at 800-228-6090 for assistance. Additional information can be found online at streck.com.

GLOSSARY OF SYMBOLS
See the Instructions (IFU) tab under Resources on the product page at streck.com.

Streck
7002 S. 109 Street, La Vista, NE 68128 USA

350731-3
2023-08