molecular designs

DOC-SPYT-INSERT-B

Streptococcus pyogenes Panel, Large Tube

Product Specification Sheet

RUO Product*

Product Description:

The *Streptococcus pyogenes* (Group A strep, GAS) panel is an *in-vitro* real-time polymerase chain reaction (RT-PCR) assay for the qualitative identification of *S. pyogenes* that is frequently found in skin and throat infections. This method is highly accurate, analytically sensitive, and is used to identify *S. pyogenes* by amplifying and detecting the genetic material.

Product Information	
S. pyogenes **Panel, Large Tube**	
Part Number	T-SPY-001-A
Number of Reactions	200
Storage Temperature	-25°C to -15°C

Product Specifications	
QC Test	qPCR Cycle Threshold Percent CV
Specification	≤ 2.5

QC Results	
Positive	meets specification
Negative	meets specification
Targets	meets specification

Disclaimer - Use of PCR and Patent

This product is for basic PCR and is outside of any valid US patents assigned to Hoffman La-Roche.

ISO Certification

This product was manufactured in a facility whose Quality Management System is certified as being in conformity with ISO 13485:2016 by Intertek.

* Limitations of Use

For Research Use Only. Not for use in diagnostic procedures.

Product Guarantee

This kit has been shown to generate reliable, repeatable and high-performance results.

Please contact Molecular Designs for technical assistance. If not completely satisfied, our team will help you identify and address the issue and replace the assays as needed.

Usage Information

Reagent Storage and Use Guidelines

1. Store all reagents at -25°C to -15°C.
2. Prior to use, thaw and store the master mix on ice.
3. Prior to use, thaw and store the primer and probe mixtures on ice.
4. Maintain the reagents on ice when in use.
5. Use reagents within couple hours of thawing.
6. Assembled reactions (master mix, primers and probes, sample) should be run within 60 minutes of assembly.
7. Reagents should not be freeze-thawed more than 4 times.

The Following is Included in the Kit:

1. 2X Master mix
2. 20X Primer and Probe mixture (Strep)
3. 20X Primer and Probe mixture (EC)
4. Nuclease Free Water
5. Positive control Template (Strep)
6. Positive control Template (EC)

The Following is Supplied by the User: Materials

1. Extracted sample(s)
2. 1.5 mL microcentrifuge tubes or equivalent
3. qPCR optical film
4. Compatible plates
5. Sealer for optical film

Equipment

1. Biosafety cabinet or laminar flow hood or PCR Dead Air Box. Do not use Laminar Flow for infectious samples
2. Pipettes and appropriate filtered pipette tips
3. Plate Vortex [recommend Vortex Genie 2 (Model G560) with the 3-inch platform and rubber cover]
4. Plate centrifuge

Instrumentation

1. CFX Touch Real-Time PCR Detection System (or equivalent)

General Guidelines and Safety Precautions

1. As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.
   a) Do not eat, drink, or smoke in designated work areas.
   b) Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples to prevent contamination. Avoid contaminating gloves when handling samples and controls.
   c) Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
   d) Thoroughly clean and disinfect all laboratory work surfaces.
   NOTE: Do not use sodium hypochlorite solution (bleach) to clean up a spill or to disinfect a plate before disposal as it can react with the common extraction reagents and generate toxic byproducts. If spills occur, follow internal procedures to immediately clean and decontaminate the surface of instrument.
2. Best practice is to use a laminar flow hood or Dead Air Box for PCR setups to reduce contamination probability. A biosafety cabinet may alternatively be used.
3. The use of filtered, sterile, and nuclease-free pipette tips is recommended.
4. False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

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Usage Information

Master Mix Preparation and Reaction Plate Setup

1. Fully thaw the master mix and primers and probe mixes on ice.
2. Gently vortex the master mix and primers and probe mixtures for least 5 seconds and centrifuge briefly to get contents to the bottom of the tubes. Keep tubes on ice.
3. Recommended reaction mixture assembly for 10 µL reactions:

Component	Volume	Final Conc.
2X Mastermix	5.0 µL	1X
20X Primers and Probe	0.5 µL	1X
DNA template	4 µL	NA
Nuclease Free Water*	0.5 µL	NA

***Use nuclease-free water for negative controls**

5. Best Practice is to prepare reaction mixture for all of the samples using the components above minus the template and dispense 6.0 µL to PCR plate.
6. Add 4.0 µL of the sample being tested to each of the target wells.
7. Add 4.0 µL nuclease-free water to the negative control well.
8. Add 4.0 µL positive sample (Strep or EC) to the positive control well.
   Note: add the same volume of PC template to the positive control well as the volume of sample being tested.
9. Seal the PCR plate using optical qPCR film.
10. Vortex the plate, at least 5 seconds per plate quadrant.
11. Spin down the plate in a plate centrifuge.

Procedural Notes

1. Do not reuse consumables. They are for one-time use only.
2. Always use caution when transferring specimens from a primary collection tube to a secondary tube.
3. Use pipettes with aerosol-barrier to handle specimens and reaction materials.
4. Always use a new pipette tip for each specimen.
5. For testing of previously frozen sample, ensure samples are equilibrated to room temperature and well mixed prior to use.

Recommended Plate Layout

For a 96-well plate, set up at least one positive and negative control. Other wells may be used for the detection of *S. pyogenes* present in the samples. Along with the *S. pyogenes* assay, 20X primers and probe mix for the Endogenous Control (extraction process control assay) is also included in the kit.

FAM is the fluorophore for both S. pyogenes and the endogenous control which will be detected on the qPCR instrument.

Usage Information

Real-Time PCR Detection System qPCR Run Setup

1. Open the specified run template and fill in the sample name fields with unique sample IDs corresponding to the samples being processed.
   NOTE: This step can also be done prior to reaction plate setup if sample IDs have already been specified.
2. Place the reaction plate into the instrument in the appropriate orientation (A1 in the upper left corner), close the instrument lid and initiate the run.

Thermocycling Protocol

1. Reverse Transcription
   a) 5 minutes at 50°C
2. Denaturation
   a) 3 minutes at 95°C
3. Annealing and Extension
   40 cycles consisting of:
   a) 5 seconds at 95°C
   b) 30 seconds at 60°C, with fluorescence acquisition during this step

Amplification Interpretation and Troubleshooting

1. The laboratory should establish cycle threshold (CT) cutoffs as appropriate for their sample workflow and procedures. It is recommended that CT cutoffs are determined during the validation of the test.
2. The laboratory should evaluate the curve shape when considering whether a sample with a given CT should be considered positive:
   a) Plate sealing issues can lead to jagged curve shapes or rising/decreasing baselines that lead to inaccurate data (erroneous CT value).
   b) Inappropriate mixing or centrifuging can lead to inaccurate data.
3. If user suspects contamination, it is recommended to clean and disinfect the laboratory area and re-test to ensure proper results.
4. A negative result for the endogenous control can be discarded if the sample is detected positive for a target assay and if the positive and negative controls have proper results.
5. Any failure of the positive or negative control should require a repeat run. If the control failure continues, it is recommended to have the qPCR instrument and the sample extraction workflow evaluated to ensure they are functioning properly.

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