appliedbiosystems

TrueMark™ STI Select Panel, Combo Kit

USER GUIDE

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

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0028493 A.0 (English)

Revision	Date	Description
A.0	11 January 2023	New document for the TrueMark™ STI Select Panel, Combo Kit.

The information in this guide is subject to change without notice.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Applied Biosystems™ TrueMark™ STI Select Panel, packaged as part of the TrueMark™ STI Select Panel, Combo Kit, is a multiplexed real-time PCR research testing solution that can detect the nucleic acid from four of the most common sexually transmitted bacterial infections and human RNase P in a single reaction well.

The panel contains primers and probe sets specific to the following targets:

- *Chlamydia trachomatis* (CT)
- *Neisseria gonorrhoeae* (NG)
- *Trichomonas vaginalis* (TV)
- *Mycoplasma genitalium* (MG)
- RNase P (human internal sample control target)

The TrueMark™ STI Select Panel, Combo Kit also includes the TrueMark™ Infectious Disease 1-Step Multiplex Master Mix (No ROX) (see page 5).

Table 1 Dyes, targets, and quenchers

Dye	Target	Quencher
FAM™ dye	*Chlamydia trachomatis* (CT)	None [1]
VIC™ dye	*Neisseria gonorrhoeae* (NG)	
ABY™ dye	*Mycoplasma genitalium* (MG)	
JUN™ dye	*Trichomonas vaginalis* (TV)	
Alexa 647 dye	RNase P (human internal sample control target)	

[1] The TrueMark™ STI Select Panel, Combo Kit probes contain QSY™ quenchers and MGB probes, which do not fluoresce. Select **None** for Quencher in the instrument set up procedure (see page 10).

For catalog numbers and storage conditions, see page 5.

IMPORTANT! It is the responsibility of the laboratories using the TrueMark™ STI Select Panel, Combo Kit to design and validate their own experimental design and analysis parameters.

Contents and storage

Table 2 TrueMark™ STI Select Panel, Combo Kit, 200 reactions (Cat. No. A57083)

Component	REF (box REF)	Amount	Storage
TrueMark™ STI Select Panel	A52691	1 × 250 µL	–30°C to –10°C
TrueMark™ Infectious Disease 1-Step Multiplex Master Mix (No ROX)	A57052	1 × 1.5 mL	–30°C to –10°C

IMPORTANT! Do not freeze-thaw the TrueMark™ STI Select Panel, Combo Kit more than 5 times.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

IMPORTANT! The customer is responsible for performing all of the necessary validations to run this assay.

Item	Source
Real-time PCR instrument and software	
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block [1]	Contact your local sales office
QuantStudio™ Design and Analysis v2.6 or later [2]	**thermofisher.com/qpcrsoftware**
Equipment	
Laboratory freezers (–30°C to –10°C)	MLS
Refrigerator (2–8°C)	MLS
BSL-2 biological safety cabinet	MLS
Centrifuge, with a rotor that accommodates standard microplates	MLS
Microcentrifuge	MLS
Laboratory mixer, vortex or equivalent	MLS

(continued)

Item	Source
Single and multichannel adjustable pipettors (2.00 µL to 1,000.0 µL)	MLS
Reagents and consumables	
TrueMark™ STI Amplification Control	A57009
Nuclease-Free Water (not DEPC-Treated)	• AM9938 (1 x 100 mL) • AM9932 (1 x 1,000 mL)
TE Buffer [3]	12090015
Reservoir for multichannel pipettes	MLS
Sterile aerosol barrier (filtered) pipette tips	**thermofisher.com/pipettetips**
MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL	• 4306737 (with barcode) • 4326659 (with barcode) • N8010560 (without barcode) • 4316813 (without barcode)
MicroAmp™ Optical Adhesive Film	4311971, 4360954
MicroAmp™ Optical Film Compression Pad[4]	4312639
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	**thermofisher.com/plastics**

[1] Ensure the system calibrations are current. Alexa Fluor™ 647 dye must be calibrated for use with this kit. Contact your local sales or service office for support.

[2] Use of QuantStudio™ Design and Analysis v2.6 or later is recommended. It is the responsibility of the laboratories using the assay to design and validate their own experimental design and analysis parameters.

[3] Low TE buffer for amplification control dilution.

[4] Recommended for use with the QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block.

2 Methods

In-use reagent stability

Reagent	Stability information
TrueMark™ STI Select Panel	Once thawed, the assay is stable for up to 8 hours at 2–8°C. Do not exceed 5 freeze-thaws.
TrueMark™ STI Amplification Control	Once thawed, the amplification control should be used immediately and returned to –30°C to –10°C storage. Do not exceed 5 freeze-thaws.
Assembled RT-PCR reaction plate	The assembled RT-PCR reaction plate is stable for up to 60 minutes at 2–8°C.

Prepare real-time PCR reactions

General laboratory recommendations

- Implement standard operating procedures in your laboratory to prevent contamination, such as the following:
  - Frequent glove changes
  - Frequent decontamination of surfaces, equipment, and pipettes with fresh 10% bleach or decontamination solution, followed by 70% ethanol
  - Use of ultraviolet light during biosafety cabinet decontamination (when available)
- Samples should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- To prevent degradation, keep master mixes, assays, and controls on ice or in cold blocks while in use.
- Do not freeze-thaw reagents more than 5 times to ensure reliable performance.
- Aliquot reagents using low DNA binding tubes to prevent stock contamination and reduce the number of freeze-thaw cycles.

- After each run, review the amplification curves for signs of inadequate vortexing or centrifugation.
- To ensure reliable performance of the real-time PCR instrument, perform preventive maintenance according to the instructions provided by the manufacturer in the instrument documentation (see page 15).

Guidelines for RT-PCR

IMPORTANT!

- Prepare the RT-PCR plate on ice or a cold block. Keep the RT-PCR plate on ice or a cold block until it is loaded into the real-time PCR instrument.
- Run the RT-PCR plate immediately. If the assembled reaction plate is not loaded into the real-time PCR instrument immediately, the plate is stable up to 1 hour at 4°C. Failure to do so could result in degraded samples.
- To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and samples, and always use aerosol barrier pipette tips.
- Maintain an RNase-free environment.
- Protect assays from light.
- Keep samples and components on ice or a cold block during use.
- For each real-time PCR plate, include the following controls:
  - Amplification control
  - No template control (NTC)

Prepare the real-time PCR reactions (96-well reaction plate)

1. If freshly extracted, put on ice. If frozen, thaw the reagents on ice or on a cold block.
2. Gently vortex the reagents, then briefly centrifuge the tube or swirl the bottle to collect the liquid at the bottom of the container. Keep on ice until use.
3. Prepare the amplification control:
   a. Pipet 495.0 µL of low TE buffer into a microcentrifuge tube, then add 5.0 µL of amplification control. Mix well, then centrifuge briefly.
   b. Pipet 432.5 µL of low TE buffer into a second microcentrifuge tube, then add 5.0 µL of the dilution created in substep 3a. Mix well, then centrifuge briefly.

   Keep on ice until use.

Note:

- This is sufficient to run 20 amplification control replicates.
- The diluted amplification control can be stored at 4-8°C and re-used within 8 hours after dilution.

4. Prepare the reaction mix:
   a. For each 96-well plate, combine the following components sufficient for the number of samples plus the amplification control and NTC.

Component	Volume per sample or control	Volume for *n* samples plus 2 controls[1]
TrueMark™ Infectious Disease 1-Step Multiplex Master Mix (No ROX)	6.25 µL	6.25 x (1.2 x *n*) µL
TrueMark™ STI Select Panel	1.25 µL	1.25 x (1.2 x *n*) µL
Total reaction mix volume	**7.50 µL**	**—**

[1] All volumes include 20% overage for pipette error.

5. Set up the reaction plate, according to the following:

   Note: Prepare the plate on ice or a cold block.

   a. Pipet 7.5 µL of the reaction mix prepared in step 4 to each well of a MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL.

   b. Add 17.5 µL of either the extracted materials, the diluted amplification control, or nuclease-free water to the designated wells .

   c. Seal the plate thoroughly with MicroAmp™ Optical Adhesive Film.

      IMPORTANT!
      - Use ONLY MicroAmp™ Optical Adhesive Film (Cat. No. 4311971, 4360954).
      - DO NOT use optical caps, MicroAmp™ Clear Adhesive Film (Cat. No. 4306311), or any other film or sealing method.
      - DO NOT heat seal the plate.
      - When applying the MicroAmp™ Optical Adhesive Film, ensure that pressure is applied across the entire plate and that there is a tight seal across every individual well. Failure to do so runs the risk of an improperly sealed well, leading to potential well-to-well contamination during vortexing and evaporation during PCR.

   d. Vortex the center of the plate for 5 seconds.

   e. Vortex each of the 4 corners of the plate for 5 seconds.

   f. Vortex the center of the plate for 5 seconds.

6. Centrifuge the reaction plate for 1-2 minutes at ≥ 650 × *g* to remove bubbles and to collect the liquid at the bottom of the reaction plate.

IMPORTANT!
- Keep the RT-PCR reaction plate at 2–8°C until it is loaded into the real-time PCR instrument immediately before starting the run.
- Run the RT-PCR reaction plate within an hour after preparation. Failure to do so could result in degraded samples.

Set up and run the real-time PCR instrument

Ensure that your real-time PCR instrument is calibrated for the dyes listed in step 6. See your instrument user guide for more information.

1. Select **Set Up Plate** from the main screen of the Design and Analysis v2.6 software.
2. In the **Systems Templates** tab, select the **Quantification-Taqman** template with the following properties:
   - **Instrument**: **QuantStudio™ 5**
   - **Block**: **96-Well 0.2-mL**
   - **Run Mode**: **Standard**
   - **Analysis**: **Standard Curve**
3. In the **Run Method** tab, set up the thermal protocol for your instrument.
   - **Reaction Volume**: **25 µL**
   - **Heater Cover Temperature**: **105.0°C**

Table 3 QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block

Step	Temperature	Time	Number of cycles	Ramp rate
UNG incubation	25°C	2 minutes	1	1.6°C per second
Reverse transcription	53°C	10 minutes	1	1.6°C per second
Preincubation	85°C	10 minutes	1	1.6°C per second
Activation	95°C	2 minutes	1	1.6°C per second
Denaturation	95°C	3 seconds	40	1.6°C per second
Anneal / extension	60°C	32 seconds		1.6°C per second

4. Set up the optical filters for your instrument based on the table below.

Table 4 Default PCR filters for the QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block

		Emission Filter					
		m1(520±15)	m2(558±11)	m3(586±10)	m4(623±14)	m5(682±14)	m6(711±12)
Excitation Filter	x1(470±15)	✔	✔	✔			
	x2(520±10)		✔	✔	✔		
	x3(550±11)			✔	✔		
	x4(580±10)				✔	✔	
	x5(640±10)					✔	
	x6(662±10)						

5. In the **Plate Setup** tab, confirm that the **Passive Reference** is set to **None**.

6. In the **Targets** table, confirm that the targets, reporter dyes, probe types, and quencher are listed correctly.

Target	Reporter dye	Probe type	Quencher
CT	FAM	MGB	None
NG	VIC		
MG	ABY		
TV	JUN		
RNase P	ALEXA 647	QSY	

IMPORTANT! Target names are case-sensitive and must be named as described.

7. Confirm that the targets in step 6 are assigned to each well in the plate layout.

8. Save the plate file and load onto the instrument.

9. Load the plate and place a MicroAmp™ Optical Film Compression Pad with the gray side down on the surface of the RT-PCR reaction plate. Ensure there is a proper seal between the thermal cycler and the adhesive film.

10. Touch **Start Run**. When prompted, confirm that you inserted a plate.

IMPORTANT!

- Be careful to place the compression pad with the brown side up and the gray side down, centered on top of the plate.
- Ensure the compression pad is free from wrinkles and signs of deterioration prior to use.
- Each compression pad may be used up to 20 times before discarding. Do not use more than 20 times. Use of deteriorated compression pads may lead to volume leakage and failed reactions.

Analyze data

IMPORTANT! It is the responsibility of the laboratories using the TrueMark™ STI Select Panel to design and validate their own experimental design and analysis parameters.

(*Recommended*) Use QuantStudio™ Design and Analysis v2.6 or later for data analysis. For more information about using the software, see page 15.

Perform qualitative analysis based on C_q values obtained from run files generated by QuantStudio™ Design and Analysis v2.6 or later.

Cross-reactivity was observed with the following organisms when using the TrueMark™ STI Select Panel:

Organism	Cross-reactive target
Pentatrichomonas hominis	*Trichomonas vaginalis*
Trichomonas tenax	*Trichomonas vaginalis*

Table 5 C_q ranges for amplification control for each target

Target	C_q range
Chlamydia trachomatis (CT)	30-36
Trichomonas vaginalis (TV)	31-35
Neisseria gonorrhoeae (NG)	30-35
Mycoplasma genitalium (MG)	30-35
RNase P (Internal control)	30-34

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Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs); found at:
  www.who.int/publications/i/item/9789240011311

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Documentation and support

Related documentation

Document	Publication Number
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
QuantStudio™ Real-Time PCR Software Getting Started Guide	4489822
QuantStudio™ Design and Analysis Software v2 User Guide	MAN0018200

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.