GeneFinder™
"SMART Solution for Better Life"

ISO 13485 Certified

STD II (MG/MH/TV) Multiplex Real-time PCR kit

REF **IFMR-04** **100 tests/ kit**

Store at -20℃ or below.
Shelf life is 12 months after manufacturing.

CE-IVD

INTENDED USE

GeneFinder™ STD II (MG/MH/TV) Multiplex Real-time PCR kit is designed for detection of DNA of *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH) and *Trichomonas viginalis* (TV) in DNA samples extracted from urogenital swabs or urine.

KIT COMPONENT

STD II (MG/MH/TV) Multiplex Real-time PCR kit	100 tests/kit
STD II Reaction Mixture*	1,050 µL
STD II Probe Mixture**	525 µL
STD II Positive Control***	50 µL
STD II Negative Control***	50 µL

*, STD II Reaction Mixture; DNA polymerase, Uracil-N-glycosidase (UNG), Buffer containing dNTPs
**, STD II Probe Mixture, Oligonucleotides for amplification and detection of target and plasmid for internal control
***, STD II Positive Control; Clones for targets
****, STD II Negative Control; Ultrapure quality water, PCR-grade

DESCRIPTION

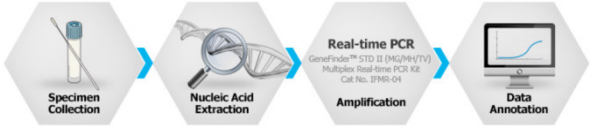
Sexually transmitted disease (STD), also referred to as sexually transmitted infections (STI) and venereal diseases (VD), are illnesses that have a significant probability of transmission between humans by means of human sexual behavior. .

GeneFinder™ STD II (MG/MH/TV) Multiplex Real-time PCR kit is a qualitative nucleic acids amplification assay and designed to detect target DNA for MG, MH and TV simultaneously.

WARNING AND PRECAUTION

- This product is designed for *in-vitro diagnostics* use Only.
- Do not use reagents past the expiration date printed on the label.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
- Unnecessary repeated freezing and thawing will be occurred inaccurate results.
- Do not mix reagent from different batches of the kit.
- Do not modify the reagent/sample volume used in the test or use in a wrong way which is not recommended.

WORK FLOW



PROTOCOL

A. Specimen

This product is must be used with DNA extracted from urogenital swab and urine.

B. DNA Extraction

It is recommended to use commercialized extraction kit such as QIAamp DNA mini kit

C. Reagent Preparation

Before setting up PCR, all components need to be thawed, gently mixed and centrifuged briefly to collect solution at the bottom.

1. Mix 10 µL of STD II Reaction Mixture and 5 µL of STD II Probe Mixture to prepare master mixture per each reaction (refer to the below). Prepare enough volume of master mixture for all the reactions plus extra to prevent pipetting error.
2. After mixing well, place 15 µL of master mixture into 96-well plate or PCR tube.
3. Add 5 µL of extracted DNA sample into tube, then mix all components by pipetting. Proceed in the same way with other DNA samples, positive and negative control (Ultrapure quality water, PCR-grade).
4. Accurately close the tube with the cap
5. Transfer the tubes or 96-well plate for test into the Real-time PCR and start for the amplification.

Component	Per reaction (µL)
STD II Reaction Mixture	10
STD II Probe Mixture	5
DNA sample or PC[1] or NC[2]	5
Total volume	20

[1], PC, positive control; [2], NC, negative control

(continued on reverse page)

D. Setting of Real-time PCR

This product is validated on Applied Biosystems 7500 Real-Time PCR instrument.

1. Referring to the instrument manual, set on the dedicated software the parameters of thermal cycle.
2. Set up the PCR program and fluorescence as following, and then click the start “Run” button.

PCR program			
Cycle		Temp.	Time
Segment 1	1 cycle	50 °C	2 min
Segment 2	1 cycle	95 °C	10 min
Segment 3	40 cycle (fluorescence scan)	95 °C	15 sec
		60 °C	60 sec

Fluorescence setting	
Target	ABI 7500
MG	FAM
MH	Texas Red
TV	JOE
Internal control (IC)	Cy5

E. Analysis Setting

Prior to the analysis, it is necessary to manually set eliminate cycle as “5” and threshold as below.

Channel (Target)	Threshold	
	Plate	Tube
FAM (MG)	100000	75000
Texas Red (MH)	100000	75000
JOE (TV)	75000	50000
Cy5 (IC)	5000	5000

F. Result Interpretation

Here are examples for result interpretation if the sample is positive / negative.

STD II		IC (Cy5)		Assay Result
Ct	Result	Ct	Result	
< 40	Pos.*	< 40	Pos.	Positive
< 40	Pos.	UD	Neg.§	Positive
UD***	Neg.**	< 40	Pos.	Negative (No Target)
< 40 (one more targets)	Pos.	< 40	Pos.	Positive (CT, NG, UU co-in fection)
UD	Neg.	UD	Neg.	Invalid§

*Pos.: Positive, **Neg.: Negative, ***UD,: Undetermined

§ When the target DNA detected in positive samples for the MG, MH and TV, the internal control (IC) may results as Ct Undetermined (UD). In fact, the low efficiency amplification reaction for the internal control may be displaced by competition with the high efficiency amplification reaction for MG, MH and TV. In such a case the sample is nevertheless suitable and the positive result of the assay is valid.

§This means that problems have occurred which may lead to incorrect results. It is not valid and needs to repeat the test.

TROUBLE SHOOTING

Problem	Possible Cause	Recommendation
No signal in all samples including positive control	Error in master mixture preparation	Check the dispensing volume during preparation of master mixture
	Inhibitors added	Repeat the extraction step with new sample
	Probe degradation	Use a new probe reagent
	Positive control degradation	Use a new positive control
	Omitted components	Verify each component, repeat the PCR mixture preparation
	Instrument setting error	Check the position setting for the positive control on the instruments. Check the thermal cycle settings on sample instrument
No signal in negative control	Carry-over contamination	Dispense carefully the sample, negative control and positive control
	96-well plate or tube error	Check the leaking of the plate or tube
	Tube cap/ sealing film error	Check the condition of closure for cap or sealing a film
	Reagent contamination	Repeat the test with new dispensing reagent
	Contamination of extraction or amplification area	Clean the instrument with disinfectant and replace with tubes and tips
Weak or no fluorescent signal in samples only	Poor DNA quality	Use recommended kit for DNA extraction and store extracted DNA at -20℃
	Insufficient volume of DNA	Repeat PCR reaction with correct volume of DNA
Weak or no fluorescent signal in positive control only	Probe degradation	Use a new probe and kit
Diverse intensity of fluorescent signals	Pipetting error	Make sure that the equal volume of reactants is added in each tube or plate
	Contamination in the outer surface of PCR tubes or plate	Wear gloves during the experiment

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