GeneFinder™
"SMART Solution for Better Life"

ISO 13485 Certified

GeneFinder™ HSV 1&2 Real*Amp* Kit

REF IFMR-19 **100 Tests/Kit**

Store at -20℃ or below.
Shelf life is 12 months after date of manufacture.

CE-IVD
Applied Biosystems 7500 Real Time PCR

INTENDED USE

The GeneFinder™ HSV 1&2 Real*Amp* Kit is designed for detection of the DNA of Herpes Simplex Virus 1 and 2 (HSV 1&2) in DNA samples.

KIT COMPONENTS

HSV 1&2 Real*Amp* Kit	**100 tests/Kit**
HSV 1&2 Reaction Mixture*	1,050 µL
HSV 1&2 Probe Mixture**	525 µL
HSV 1&2 Positive Control***	50 µL
HSV 1&2 Negative Control***	50 µL

* HSV 1&2 Reaction Mixture; DNA polymerase, Uracil-N-glycosidase (UNG), Buffer containing dNTPs
** HSV 1&2 Probe Mixture, Oligonucleotides for amplification and detection of target and plasmid for internal control
*** HSV 1&2 Positive Control; Clones for targets
**** HSV 1&2 Negative Control; Ultrapure quality water, PCR-grade

DESCRIPTION

The herpes simplex virus, or herpes, is categorized into 2 types: herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2).

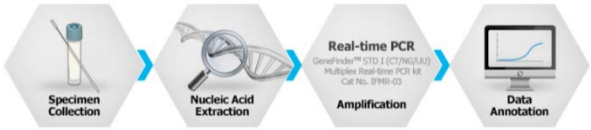
HSV-1 is mainly transmitted by oral to oral contact to cause infection in or around the mouth (oral herpes). HSV-2 is almost exclusively sexually transmitted, causing infection in the genital or anal area (genital herpes).

The GeneFinder™ HSV 1&2 Real*Amp* Kit is a qualitative nucleic acids amplification assay designed to detect target DNA for HSV 1&2 simultaneously.

WARNINGS AND PRECAUTIONS

- 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 lead to 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 incorrectly—in a way which is not recommended.

WORKFLOW



PROTOCOL

A. Specimen

This product is must be used with DNA extracted from whole blood, plasma or genital swabs samples..

B. DNA Extraction

Use of a commercialized extraction kit, such as the QIAamp DNA Blood Mini Kit, is recommended.

C. Reagent Preparation

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

1. Mix 10 µL of HSV 1&2 Reaction Mixture and 5 µL of HSV 1&2 Probe Mixture to prepare master mixture per each reaction (refer to the table below). Prepare enough volume of master mixture for all the reactions, plus 10% 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, and 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 testing into the Real-time PCR and start the amplification.

Component	**Per Reaction (µL)**
HSV 1&2 Reaction Mixture	10
HSV 1&2 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 up Real-time PCR

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

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

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

Fluorescence Setting		
Target	Reporter	Quencher
HSV 1	FAM	None
HSV 2	VIC	None
Internal control (IC)	Cy5	None
Passive dye	ROX	

E. Analysis Setting

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

Channel (Target)	Threshold
FAM (HSV 1)	0.1
VIC (HSV 2)	0.1
Cy5 (IC)	0.05

F. Result Interpretation

Here are examples for interpretation of results (for both positive and negative samples).

HSV 1 & 2		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 (HSV 1&2 co-infection)
UD	Neg.	UD	Neg.	Invalid$

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

§ When the target DNA is detected in samples as positive for HSV 1 and 2, the internal control (IC) may show result as Ct Undetermined (UD). In fact, the low-efficiency amplification reaction for the internal control may be displaced by competition from the high-efficiency amplification reaction for HSV 1 and HSV 2. 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. The test is not valid and needs to be repeated.

TROUBLESHOOTING

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 for 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

Please refer to Full Manual for details.

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