GeneFinder™
"SMART Solution for Better Life"

OHC OSANG HEALTHCARE

HLA-B*51 Real*Amp* Kit

REF **IFMR-09.02B100** Σ **100 Tests/ Kit**

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

CE-IVD (CE0123)

Applied Biosystems 7500 Real Time PCR
Bio-Rad CFX96 Real Time PCR

INTENDED PURPOSE

GeneFinder™ HLA-B*51 Real*Amp* kit is for in vitro diagnostic medical device to aid diagnosis of HLA-B*51 allele associated disease.
GeneFinder™ HLA-B*51 Real*Amp* kit is a qualitative by using Real-time polymerase chain reaction (RT-PCR) to detect HLA-B*51 allele in the genomic DNA extracted from whole blood collected from individuals with suspected Behcet's disease.

INTENDED USER

GeneFinder™ HLA-B*51 Real*Amp* Kit is intended for use by qualified clinical laboratory personnel (Professional Use Only) specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

KIT COMPONENT

HLA-B*51 Real*Amp* Kit	**100 Tests**
B51 2X *Rxn**	550 ㎕
B51 DNA pol.**	110 ㎕
B51 PC***	50 ㎕

*, B51 2X *Rxn,* Oligonucleotides for amplification and detection of target and internal control, Buffer containing dNTPs.
**, B51 DNA pol., DNA polymerase.
***, B51 PC, Clones for targets.

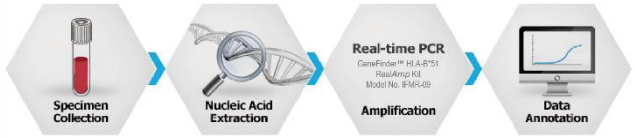
DESCRIPTION

The human leukocyte antigen (HLA) is well known as genetic factor for disease sensitivities and plays a key role in transplantations, transfusions and parentage test. Behcet's disease (BD) is chronic inflammatory disorder, involving several organs. Although the exact pathogenesis for BD is not completely understood, it has been suggested that the disease is triggered in genetically susceptible individuals by environmental factors, such as microbial agents.
According to the reports, HLA-B*51 alleles which are responsible for immune function of human leukocyte antigen have been reported that patients of Behcet's disease were observed highly concentration than normal. GeneFinder™ HLA-B*51 Real*Amp* kit is a qualitative assay using real-time PCR, and highly reliable kit.

WARNING AND PRECAUTION

- This product is designed for *in-vitro diagnostics use Only*.
- This product should be used by *professionals in the laboratory*.
- Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local regulations.
- Follow universal precautions when performing the assay.
- Handle samples as if capable of transmitting infection.
- Unnecessary repeated freezing and thawing will be occurred inaccurate results.
- Store the kit away from any source of contamination DNA, especially amplifies nucleic acid.
- Do not use a kit after expiry date.
- Do not mix reagent from different lot.
- Do not modify the reagent or sample volume used in the test or use in a wrong way which is not recommended.
- The test results provided by the product should be interpreted with clinical result. It is user's responsibility for erroneous results due to experimental method which is not recommended by the manufacturer.

WORK FLOW



PROTOCOL

A. Specimen

This product has to be used for genomic DNA samples extracted from blood (with EDTA). Do not use blood sample in heparin which can lead to PCR reaction inhibition.

B. DNA Extraction

It is recommended to use commercialized extraction kit such as QIAamp DNA blood 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 5 ㎕ of B51 2X *Rxn* and 1 ㎕ of B51 DNA pol. 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 6 ㎕ of master mixture into 96-well plate or PCR tube.
3. Add 4 ㎕ of extracted DNA sample into 96-well plate or 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).
   §, For CFX96, it is recommended to use BR white plate/tube for the best result.
4. Accurately close the tube with the cap or seal the 96-well plate.
5. Transfer the tubes or 96-well plate for test into the real-time PCR and start for the amplification.

Component	**Per reaction (㎕)**
B51 2X *Rxn*	5
B51 DNA pol.	1
DNA sample or PC or NC	4
Total volume	10

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

(continued on reverse page)

D. Setting of Real-time PCR

This product is validated on ABI 7500 Real-Time PCR instrument system and Bio-Rad CFX96 Real-Time PCR instrument system.

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				
	Step	**Temp.**	**Time**	**Cycle**
1	Denaturation	96 °C	5 min	1
2	Denaturation	96 °C	25 sec	5
	Annealing	70 °C	45 sec	
	Extension	72 °C	30 sec	
3	Denaturation	96 °C	25 sec	30
	Annealing ¶	65 °C	45 sec	
	Extension	72 °C	30 sec	

¶, Instrument setting; **for ABI7500**, select ON for data collection; **for CFX96**, select ‘Add plate Read to Step’.

Fluorescence setting		
Target	**Instrument**	
	ABI 7500	**CFX96**
HLA-B*51	FAM	FAM
Internal control (IC)	Cy5	Cy5
Passive dye	ROX	-

E. Analysis Setting

The values of fluorescence emitted by the specific probes and by the specific internal control probe in amplification reactions should be analyzed by the instrument software.

1. Prior to result analysis, set the baseline and threshold as the below.
2. Start to analyze the result.

Instrument	**Baseline setting**		**Threshold setting**	
	B*51	**IC**	**B*51**	**IC**
ABI 7500	6~15		0.1	0.1
CFX96	6~15		500	300

F. Result Interpretation

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

Ct value			
B*51 (FAM)	**IC (Cy5)**	**Result**	**Remark**
16-26	≤ 28	B*51 positive	-
16-26	> 28 or UD	B*51 positive	If Ct value of IC is out range of criteria and target Ct value is within the criteria, a sample is positive for B*51 allele.
UD§	≤ 28	B*51 negative	-
UD	UD	Invalid	Repeat the test from DNA ex-traction step.

§, UD,: Undetermined

QUALITY CONTROL

- PC and NC Ct range should be as below:

#	**Ct value**	
	B*51 (FAM)	**IC (Cy5)**
PC	18±3	≤ 28
NC	UD	UD

Trouble shooting

Problem	**Possible Cause**	**Recommendation**
No fluorescent signal is detected 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 2X *Rxn* 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
Non-Specific fluorescent signal is detected in negative control	Carry-over contamination	Beware when dispensing sample, ultrapure water and positive control
	96-well plate or tube error	Beware of spilling the contents 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 positive control
Diverse intensity of fluorescent Signals appear	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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