GeneFinder™
"SMART Solution for Better Life"

OHC OSANG HEALTHCARE

HLA-B*57:01 Real*Amp* Kit

REF **IFMR-22.02B050** **50 Tests / Kit**

The kit must be stored below -20℃.
Shelf life is 12 months after manufacturing.

CE-IVD (CE0123)

Applied Biosystems 7500 Real-Time PCR system
Applied Biosystems 7500 Fast Real-Time PCR system
Bio-Rad CFX96 Real-Time Detection system

INTENDED USE

GeneFinder™ HLA-B*57:01 Real*Amp* kit is *in vitro diagnostic* Kit and qualitative by using Real-time polymerase chain reaction (RT-PCR) to detect HLA-B*57:01 allele in the genomic DNA extracted from whole blood collected from individuals with identify patients at risk for hypersensitivity to abacavir.

KIT COMPONENT

HLA-B*57:01 Real*Amp* Kit	50 Tests / Kit
B5701 *Rxn**	750 ㎕
B5701 DNA pol.**	50 ㎕
B5701 PC***	50 ㎕

*, B5701 *Rxn,* Oligonucleotides for amplification and detection of targets and internal control, Buffer containing dNTPs.
**, B5701 DNA pol., DNA polymerase.
***, B5701 PC, B5701 Positive Control.

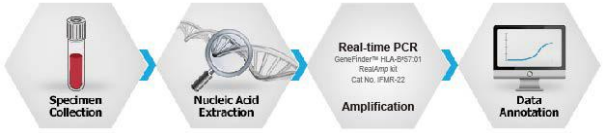
DESCRIPTION

Abacavir is a nucleoside reverse-transcriptase inhibitor with activity against the human immunodeficiency virus (HIV). The abacavir hypersensitivity reaction is a multiorgan clinical syndrome typically seen within the initial 6 weeks of abacavir treatment.
In approximately 5-9% Caucasian of treated patients, abacavir can induce an immune mediated hypersensitive response that correlates with the presence of the HLA-B* 57:01 allele. Consequently, current guidelines recommend screening for the presence of the HLA-B*57:01 allele in all HIV infected patients before abacavir initiation.

GENERAL SAFETY INSTRUCTIONS

- This product should be used by professionals in the laboratory.
- Read the instructions in the package carefully before processing samples.
- Do not use past the expiration date.
- Use aerosol-resistant pipette tips and use a new tip every time a volume is dispensed.
- Store the regents recommended temperature.
- Do not mix reagent from different lot.
- Do not modify the reagent/sample volume used in the test or use in a wrong way which is not recommended.
- It is user's responsibility for erroneous results due to experimental method which is not recommended by the manufacturer.

WORK FLOW



PROTOCOL

A. Specimen
GeneFinder™ HLA-B*57:01 RealAmp kit is used with genomic DNA samples extracted from blood (EDTA). Do not use blood sample in heparin which can lead to PCR reaction inhibition.

B. DNA Extraction
It is recommended using commercial DNA extraction kit such as Qiagen DNA blood mini kit (Qiagen, Germany, Cat # 51104).

C. DNA Quality
The recommended concentration of DNA applied to this kit is approximately 25-100 ng/㎕ and DNA A260/280 ratio is within 1.6 ~2.0.

D. 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 15 µl of B5701 *Rxn* and 1 µl of B5701 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 each 16 µl of master mixture into 96-well plate or PCR tube.
3. Add 4 µl 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 control(B5701 PC), No Template Control (water).
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 test (µl)
B5701 *Rxn*	15
B5701 DNA pol.	1
DNA sample or PC or NTC	4
Total volume	**20**

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E. Setting of Real-time PCR

This product is validated on
Applied Biosystems 7500 Real-Time PCR system,
Applied Biosystems 7500 Fast Real-Time PCR system,
Bio-Rad CFX96 Real-Time Detection system.

1. Referring to the instrument manual, set on the dedicated software the parameters of thermal cycle.
2. Ramp speed:
   - ABI7500 & ABI7500 Fast :
   √ **Standard** (~2 hours to complete a run)
3. 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 & ABI7500 Fast, select ON for data collection.
for CFX96, select 'Add plate Read to Step'.

Fluorescence setting

Target	Instrument	
	ABI	CFX96
HLA-B*57:01	FAM	FAM
HLA-B*57	JOE	HEX
Internal control (IC)	ROX	Texas Red

F. Analysis Setting

The values of fluorescence emitted by the specific target 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	Threshold			Baseline	
	FAM	JOE/ HEX	ROX/ Texas Red	Start	End
ABI 7500	30,000			6	15
ABI 7500 Fast					
CFX96	300	500	200		

G. Result Interpretation

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

Ct value			Result	Remark
FAM	JOE/ HEX	ROX/ Texas Red		
16-26	16-26	≤ 28	HLA-B*57:01 Positive	-
16-26	16-26	UD§ or > 28	HLA-B*57:01 Positive	If Ct value of IC is out range of criteria and target Ct value is within The criteria, a sample is positive for target allele
<16 or >26 or UD	16-26	≤ 28	HLA-B*57:01 Negative	HLA-B*57 subtype alleles
<16 or >26 or UD	<16 or >26 or UD	≤ 28	HLA-B*57:01 Negative	Not included HLA-B*57 subtype alleles
16-26	<16 or >26 or UD	≤ 28	HLA-B*57:01 Negative	Not included HLA-B*57 subtype alleles
<16 or >26 or UD	<16 or >26 or UD	UD or > 28	Invalid	Repeat the test from DNA extraction step.

§, UD,: Undetermined

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	Take care when DNA is extracted and repeat the extraction step with new sample
	Probe degradation	Use a new B5701 *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 no template control	Carry-over contamination	Beware when dispensing sample, ultrapure water and positive control
	Microplate/ tube error	Beware of spilling the contents of the plate or tube
	Tube cap/ sealing film error	Beware of closing a cap or sealing a film
	Reagent contamination	Repeat the test with new dispensing reagent
	Contamination of extraction/ amplification area	Clean the instrument with disinfectant and replace with tubes and tips
Weak or absence fluorescent intensity 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 absence fluorescent intensity in positive control only	Positive control degradation	Use a new positive control
Diverse intensity of fluorescent signals appear	Pipetting error	Make sure that the equal volume of reactants are 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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