PRODUCT DATA SHEET

HLA B*5701 Real-TM

Handbook

Real Time PCR test for the detection of HLA-B

(major histocompatibility complex, class I, B) Allele 5701

REF H53-100FRT

NAME

HLA B*5701 Real-TM

INTRODUCTION

Abacavir is a nucleoside reverse-transcriptase inhibitor with activity against the human

immunodeficiency virus (HIV), available for once-daily use in combination with other

antiretroviral agents, that has shown efficacy, few drug interactions, and a favorable long-term

toxicity profile. The most important adverse effect of abacavir that limits its use in therapy and

mandates a high degree of clinical vigilance is an immunologically mediated hypersensitivity

reaction affecting 5 to 8% of patients during the first 6 weeks of treatment. Symptoms of a

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

hypersensitivity reaction to abacavir are nonspecific and include combinations of fever, rash,

constitutional symptoms, gastrointestinal tract symptoms, and respiratory symptoms that become

more severe with continued dosing. Immediate and permanent discontinuation of abacavir is

mandated, resulting in a rapid reversal of symptoms. Subsequent rechallenge with abacavir is

contraindicated, since it can result in a more severe, rapid, and potentially life-threatening

reaction. In 2002, an association between a diagnosis of hypersensitivity reaction to abacavir and

carriage of the major histocompatibility complex class I allelee HLA-B*5701 was reported

independently by several independent studies.

Studies of cohorts with HIV infection have also shown that avoiding abacavir in HLA-B*5701.

positive patients significantly reduced the incidence of suspected hypersensitivity reaction up to

0,5%. Many clinical studies recommend for this reason, the pharmacogenetic molecular testing

of the carriage of the major histocompatibility complex class I allelee HLA-B*5701 in all HIV

positive patients treated with abacovir.

HLA-B*5701 Real-TM test can predict who will develop a severe allergic reaction to the anti-

HIV drug abacavir as the presence of HLA-B*5701 is significantly associated with an abacavir

hypersensitivity.

INTENDED USE

HLA B*5701 Real-TM is a Real-Time amplification test for the detection of HLA-B (major

histocompatibility complex, class I, B) Allele 5701 in the biological materials.

The kit HLA B*5701 Real-TM can be used as screening test for the prevention of abacavir

hypersensitivity reactions.

PRINCIPLE OF ASSAY

HLA B*5701 Real-TM Test is based on two major processes: isolation of genomic DNA from

specimens and Real Time amplification with allele specific primers. The real-time PCR

monitoring of fluorescence intensities allows the accumulating product detection without

reopening of reaction tubes after the PCR run. HLA B*5701 Real-TM PCR kit is a qualitative

test which contains the Internal Control IC (human beta-globine gene), which allows to control

the presence of cellular material in the sample.

MATERIALS PROVIDED

Contents Ref. H53-100FRT

100 reactions

PCR-mix-1 HLA 2 x 0,6 ml

PCR-buffer-FRT 2 x 0,3 ml

TaqF Polymerase 2 x 0,03 ml

Negative Control (C-)* 4 x 0,5 ml

TE-buffer 2 x 0,07 ml

Pos C+ (HLA B*5701 & human DNA) 1 x 0,2 ml

*** must be used in the isolation procedure as Negative Control of Extraction.**

MATERIALS REQUIRED BUT NOT PROVIDED

E DNA isolation kit

E Desktop microcentrifuge for geppendorfh type tubes

E Vortex mixer

E Disposable gloves, powderless

E Biohazard waste container

E Refrigerator, Freezer

E Real Time Thermal cycler

E Workstation

E Pipettes (adjustable)

E Sterile pipette tips with filters

E Tube racks

WARNINGS AND PRECAUTIONS

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and

reagents. Thoroughly wash hands afterward.

2. Do not pipette by mouth.

3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.

4. Do not use a kit after its expiration date.

5. Do not mix reagents from different kits.

6. Dispose of all specimens and unused reagents in accordance with local regulations.

7. Specimens and controls should be prepared in a laminar flow hood.

8. Heparin has been shown to inhibit reaction. Use of heparinized specimens is not

recommended.

9. Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the

test.

10. Once the reagents have been thawed, vortex and centrifuge briefly the tubes.

11. Prepare quickly the Reaction mix on ice or in the cooling block.

12. Specimens may be infectious. Use Universal Precautions when performing the assay.

13. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5%

sodium hypochlorite, or other suitable disinfectant. Follow by wiping down the surface with

70% ethanol.

14. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If

these solutions come into contact, rinse immediately with water and seek medical advice

immediately.

15. Material Safety Data Sheets (MSDS) are available on request.

16. Use of this product should be limited to personnel trained in the techniques of DNA

amplification.

17. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the

Extraction Area and moving to the Amplification Area. Do not return samples, equipment

and reagents in the area where you performed previous step. Personnel should be using

proper anti-contamination safeguards when moving between areas.

STORAGE INSTRUCTIONS

The kit HLA B*5701 Real-TM must be stored at -20‹C.

The kit can be shipped at 2-8‹C for 3-4 days but should be stored at -20‹C immediately on

receipt.

STABILITY

HLA B*5701 Real-TM is stable up to the expiration date indicated on the kit label. The product

will maintain performance through the control date printed on the label. Exposure to light, heat

or humidity may affect the shelf life of some of the kit components and should be avoided.

Repeated thawing and freezing of these reagents should be avoided, as this may reduce the

sensitivity.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

HLA B*5701 Real-TM can analyze genomic DNA extracted from:

E whole blood collected in EDTA tubes;

Specimens can be stored at +2-8‹C for no longer than 48 hours, or freeze at -20 ‹C to80 ‹C.

Transportation of clinical specimens must comply with country, federal, state and local

regulations for the transport of etiologic agents.

DNA ISOLATION

The following isolation kits are recommended:

Ë Genomic column DNA Express . spin column extraction kit (Sacace, REF K-1-1/E)

Ë QIAamp DNA Blood mini kit (Qiagen, 51104)

DNA samples with concentration in range from 20 to 200 ng/ƒÊl could be analyzed.

REAGENT PREPARATION

1. Prepare the required quantity of reaction tubes for samples (N) and controls (N+2).

2. Prepare Reaction Mix by adding for each sample into the new sterile tube 10*N ƒÊl of PCRmix-

1 HLA and 5*N ƒÊl of PCR-buffer-FRT and 0,5*N ƒÊl of TaqF Polymerase. Vortex

and centrifuge for 2-3 sec.

3. Add to each reaction tube 15 ƒÊl of Reaction Mix and 10 ƒÊl of extracted genomic DNA. Mix

by pipetting.

4. Prepare for each panel 2 controls:

□œ add 10 ƒÊl of TE-buffer to the tube labeled Amplification Negative Control;

□œ add 10 ƒÊl of Pos C+ to the tube labeled Amplification Positive Control;

5. Insert the tubes in the thermalcycler.

Create a temperature profile on your Real-time instrument as follows:

Rotor type instruments1 Plate type or modular instruments2

Stage „Semp, □‹„R Time

Fluorescence

detection

Cycle

repeats

„Semp,□‹„R Time Fluorescence detection

Cycle

repeats

Hold 95 15 min . 1 95 15 min . 1

Cycling

95 5 s .

5

95 5 s .

5

60 20 s . 60 20 s .

Cycling 2

95 5 s .

40

95 5 s .

40

60 40 s

FAM(Green),

JOE(Yellow)

60 50 s FAM, JOE/HEX/Cy3

1 For example Rotor-Gene. 3000/6000 (Corbett Research, Australia)

2 For example, iQ5./iQ iCycler. (BioRad, USA); Mx3000P/Mx3005P. (Stratagene, USA), Applied

BiosystemsR 7300/7500 Real Time PCR (Applera), SmartCyclerR (Cepheid)

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RESULTS ANALYSIS

The results are interpreted by the device software through the presence of crossing of

fluorescence curve with the threshold line.

DNA HLA*B5701 is detected on the JOE (Yellow)/HEX/Cy3 channel and IC on the FAM

(Green) channel.

Results are accepted as relevant if both positive and negative controls of amplification along

with negative control of extraction are passed (see table 1).

Table 1. Results for controls

Control Stage for control Ct channel Fam (Green) Ct channel Joe

(Yellow)/ HEX/Cy3 Interpretation

NCS DNA isolation Neg Neg Valid result

TE-buffer Amplification Neg Neg Valid result

Pos C+ Amplification Pos Pos Valid result

E The sample is considered to be positive if in the channel Joe (Yellow)/HEX/Cy3 the

result is positive and the value of Ct on this channel is higher than Ct on the Fam (Green)

channel but not more than 5 cycles.

E The sample is considered to be negative if in the channel Joe (Yellow)/HEX/Cy3 value is

negative or if the value of Ct on this channel is higher than Ct on the Fam (Green) of

more than 5 cycles.

E Normal difference between Joe (Yellow) and Fam (Green) Ct values is 2-3 cycles.

TROUBLESHOOTING

1. Absent signal of the IC (Fam (Green) channel): retesting of the sample is required.

E The PCR was inhibited.

Ë Make sure that you use a recommended DNA extraction method and follow

the manufacturer□fs instructions.

□E The reagents storage conditions didn□ft comply with the instructions.

□Ë Check the storage conditions

□E The PCR conditions didn□ft comply with the instructions.

□Ë Check the PCR conditions and for the IC detection select the fluorescence channel reported in the protocol.

□E No correct sample collection or preparation.

2. No signal on the Joe (Yellow)/Cy3/HEX and Fam (Green) channels with Positive Control.

□E The reagents storage conditions didn□ft comply with the instructions.

□Ë Check the storage conditions

□E The PCR conditions didn□ft comply with the instructions.

□Ë Check the temperature profile and select the fluorescence channel reported in the protocol.

□E Incorrect configuration of the PCR reaction:

□Ë Check the reagents preparation step.

3. Any signal with Negative Control.

□E Contamination during PCR preparation procedure. All samples results are invalid.

□Ë Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.

□Ë Pipette the Positive controls at the end.

□Ë Repeat the PCR preparation with the new set of reagents.

4. Variation of more than 5 cycles between Ct values of Fam(Green) and Joe (Yellow) in a

sample.

E Contamination with genomic material during PCR preparation procedure. Sample

result is invalid, retesting of sample is required.

PERFORMANCE CHARACTERISTICS

The analytical sensitivity

The analytical sensitivity was determined using Company standard sample POS HLA B*5701

which is a plasmid pGEM-t solution containing DNA fragment of allele HLA B*5701 (2nd and

3rd exon of HLA B*5701 gene) with a known concentration. The sample was tested using real

time PCR with its dilutions 10x and 2x in standard DNA buffer.

The analytical sensitivity of Sacace HLA B*5701 Real-TM kit was declared as 1000 copies/ml

(positive result in a 12 times tested sample with this concentration).

The analytical specificity

The analytical specificity was valued using DNA controls (13 samples) with a known allelelic

combination of HLA previously tested using sequence specific primers.

DNA CONTROLS

No. Sample Allele HLA-B

1 R98-149776Z 5704 5301

2 R97-403860J 5701 801

3 R98-117211A 5701 3701

4 R98-106525C 4201 5801

5 R98-136122N 5801 1502

6 R98-1036872Z 4403 4001

7 R98-278853J 15 8

8 R98-101966H 5701 801

9 R97-397992J 44 40

10 R90 50043X 35 61

11 R91 7077Y 51 55

12 BSN 9400419 5101 5703

13 R92 20601H 27 40

DNA samples performed on RotorGene 6000

Samples containing

HLA B*5701allele

Amplification of

s-globine gene (IC)

The results of the analysis of DNA samples show the absence of a cross reaction with above

mentioned alleles, especially belonging to B*57 group (B*5703 and B*5704).

REPRODUCIBILITY

Reproducibility was tested in two indipendent assays using different dilutions of company

standard sample POS HLA B*5701

Test

Concentration,

copies/ml

Pos/total Medium Ct Dev Ct

1

2 * 107 4/4 15,73 0,12

2 * 106 4/4 18,91 0,14

2 * 105 4/4 22,28 0,12

2 * 104 4/4 25,90 0,24

2 * 103 4/4 28,01 0,29

1 * 103 4/4 39,25 0,36

2

2 * 107 3/3 15,40 0,11

2 * 106 3/3 18,58 0,19

2 * 105 3/3 22,93 0,18

2 * 104 3/3 25,56 0,24

2 * 103 3/3 28,78 0,27

1 * 103 9/9 29,93 0,37

No. Sample Allele HLA-B

Results with

Sacace. HLA B*5701 Real-TM kit

HLA B*5701 „B„K„O

1 R98-149776Z 5704 5301 - +

2 R97-403860J 5701 801 + +

3 R98-117211A 5701 3701 + +

4 R98-106525C 4201 5801 - +

5 R98-136122N 5801 1502 - +

6 R98-1036872Z 4403 4001 - +

7 R98-278853J 15 8 - +

8 R98-101966H 5701 801 + +

9 R97-397992J 44 40 - +

10 R90 50043X 35 61 - +

11 R91 7077Y 51 55 - +

12 BSN 9400419 5101 5703 - +

13 R92 20601H 27 40 - +

Diagnostic sensitivity and specificity

The method of direct sequencing was used as reference because no other commercial certified kit

was found.

Results with

Sacace. HLA B*5701 Real-TM kit

+ -

Direct

sequencing

+ 34 0

- 0 317

The experimental group included 351 patients were tested both by direct sequencing method and

by real time PCR using Sacace. HLA B*5701 Real-TM kit. A complete correspondence was

observed between both methods showing a diagnostic sensitivity and specificity of Sacace HLA

B*5701 Real-TM kit to 100%.

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EXPLANATION OF SYMBOLS

REF Catalogue Number

RUO Research Use Only

LOT Lot Number

Expiration Date

Contains reagents

Caution!

VER Version

Manufacturer

Temperature limitation

****iCycler. and iQ5. are trademarks of Bio-Rad Laboratories**

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Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

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Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium