CE 1023

IVD For *in Vitro* Diagnostic Use

For Professional Use Only

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

Σ 100

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 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 PCR kit is an in vitro nucleic acid amplification test for qualitative detection of B locus 5701 allele of human major histocompatibility complex (HLA B*5701) in the clinical material (whole blood and oropharyngeal swabs) using real-time hybridization-fluorescence detection of amplified products.

The results of PCR analysis are taken into account in complex diagnostics of disease.

PRINCIPLE OF PCR DETECTION

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

Reagent	*Description*	*Volume, ml*	*Amount*
PCR-mix-1-FRT HLA	colorless clear liquid	0.6	2 tubes
RT-PCR-mix-2-FL	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
TE-buffer	colorless clear liquid	0.07	2 tubes
Positive Control DNA HLA B*5701 and human DNA (C+)	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	0.5	4 tubes

Contains reagents for 110 tests.

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

MATERIALS REQUIRED BUT NOT PROVIDED

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers.
- Disposable polypropylene 1,5/2,0 ml tubes.
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 1,5/2,0 ml tubes.
- PCR Workstation.
- Real Time Thermal cycler.
- Disposable polypropylene microtubes for PCR.
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

QUALITY CONTROL

In accordance with Sacace's ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

WARNINGS AND PRECAUTIONS

IVD ***In Vitro* Diagnostic Medical Device**

For *In Vitro* Diagnostic Use Only

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. Dispose of all specimens and unused reagents in accordance with local regulations.
6. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
7. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
8. 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.
9. Material Safety Data Sheets (MSDS) are available on request.
10. Use of this product should be limited to personnel trained in the techniques of DNA amplification.
11. PCR reactions are sensitive to contamination. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practice.
12. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the PCR and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

Sampling of biological materials for PCR-analysis, transportation, and storage are described in details in the handbook of the manufacturer. It is recommended that this handbook is read before beginning of the work.

STORAGE INSTRUCTIONS

The components of **HLA B*5701 Real-TM** must be stored at -16°C or below when not in use. The kit can be shipped at 2-8°C for no longer than 5 days but should be stored at -16°C or below 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 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.

The shelf life of reagents before and after the first use is the same, unless otherwise stated.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

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

- *whole blood:* Collect 2 ml of blood to a tube with 0.2 ml of 3% EDTA solution. Invert a closed tube several times to ensure proper mixing. Blood samples should be stored at 2–8 °C for up to 48 h
- *Oropharyngeal swabs* are taken with a sterile probe with a cotton tip. After swabbing, the probe should be placed to a tube with 0.5 ml of “Transport Medium for Storage and Transportation Respiratory Swabs” (REF 958). The probe should be broken off at the score mark so that the tube is tightly closed. The sample should be stored at 2–8 °C for up to 3 days.

Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

DNA ISOLATION

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the “SAMPLE COLLECTION, STORAGE AND TRANSPORT” paragraph, could be used.

Sacace Biotechnologies recommends to use the following kits:

⇒ **DNA/RNA-Prep** (Sacace, REF K-2-9).

Please carry out the DNA extraction according to the manufacturer’s instructions.

REAGENTS PREPARATION (REACTION VOLUME 25 µL):

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

Unfreeze reagents before mixing.

1. Prepare the required number of the tubes for amplification of DNA from clinical and control samples.
2. Prepare in a new sterile tube the **Reaction Mix.** For each sample mix **10*(N+1) µl** of **PCR-mix-1-FRT HLA, 5.0*(N+1) µl** of **RT-PCR-mix-2-FL** and **0.5*(N+1) µl** of **Polymerase (TaqF).** Vortex the tube, then centrifuge shortly.
3. Add **15 µl** of **Reaction Mix** and **10 µl** of **extracted DNA** sample to appropriate tube. Mix by pipetting.
4. Carry out the control amplification reactions:

NCA - Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ - Add **10 µl** of **Positive Control DNA HLA B*5701 and human DNA** to the tube labeled C+ (Positive Control of Amplification).

5. Insert the tubes in the thermalcycler

Amplification

Create a temperature profile on your instrument as follows:

Step	Rotor type instruments[1]			Plate type or modular instruments[2]		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
Hold	95	15 min	1	95	15 min	1
Cycling	95	5 sec	5	95	5 sec	5
	60	20 sec		60	20 sec	
Cycling 2	95	5 sec	40	95	5 sec	40
	60	40 sec **fluorescence detection**		60	50 sec **fluorescence detection**	

1 For example, Rotor-Gene™ 3000/6000 (Qiagen) or equivalent.
2 For example, iCycler iQ5™ (BioRad) or equivalent.

Fluorescence is detected at the 2nd step of Cycling 2 stage (60 °C) in FAM/Green and JOE/Yellow/HEX/Cy3, fluorescence channels.

INSTRUMENT SETTINGS (Rotor-Gene 6000/Q)

Rotor-type instruments

Channel	Calibrate/Gain Optimisation…	Threshold	More Settings/ Outlier Removal	Slope Correct
FAM/Green	*from 5 Fl to 10 Fl*	*0.03*	*10 %*	*On*
JOE/Yellow	*from 5 Fl to 10 Fl*	*0.03*	*20 %*	*On*

Plate- or modular type instruments

The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold at a level where fluorescence curves are linear and do not cross curves of the negative samples.

DATA ANALYSIS

- **HLA*B5701** DNA is detected on JOE (Yellow)/HEX/Cy3 channel,
- **IC (**human beta-globine gene) on FAM (Green) channel.

RESULTS INTERPRETATION

The results are interpreted by the device software by the crossing (or not crossing) of the fluorescence curve with the threshold line.

The results of the analysis are considered reliable only if the results obtained for both Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct.

Results for controls

Sample	Stage for control	Ct in channel				Interpretation
		RotorGene		Plate Type		
		Green	Yellow	FAM	JOE/HEX	
NCA	Amplification	Neg	Neg	Neg	Neg	OK
C+	Amplification	Pos (< 25)	Pos (< 25)	Pos (< 29)	Pos (< 29)	OK

- The sample is considered to be positive if in the Joe (Yellow)/HEX/Cy3 channel 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 (see table 2).
- 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.
- Normal difference between Joe (Yellow) and Fam (Green) Ct values is 2-3 cycles.

Results for samples

Sample	Ct value and result			
	RotorGene		Plate Type	
	FAM, IC	JOE, HLA	FAM, IC	HEX, HLA
C+	< 25 (positive)	< 25 (positive)	< 29 (positive)	< 29 (positive)
Clinical sample	< 25 (positive)	< Ct (FAM)+5 (positive)	< 29 (positive)	< Ct (FAM)+5 (positive)

PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity of **HLA B*5701 Real-TM** PCR kit is $1x10^3$ cells/ml.

The claimed analytical features of **HLA B*5701 Real-TM** PCR kit are guaranteed only when additional reagent kit DNA/RNA-prep (Sacace, REF K-2-9) is used.

Specificity

The analytical specificity of **HLA B*5701 Real-TM** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. The clinical specificity of **HLA B*5701 Real-TM** PCR kit was confirmed in laboratory clinical trials.

Target region

Channel for fluorophore	FAM	JOE
DNA-target	β-globin gene	alleles 5701 of B gene
Target gene	β -globin gene	alleles 5701 of B gene

QUALITY CONTROL PROCEDURE

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. If the sample is not correctly prepared or it is an insufficient quantity of epithelial cells the Internal Control will not be detected.

A negative amplification control (NCA), positive amplification control (C+) are required for every run to verify that the specimen preparation, the amplification and the detection steps are performed correctly.

If the controls are out of their expected range (see table Results for Controls), all of the specimens and controls from that run must be processed beginning from the sample preparation step.

TROUBLESHOOTING

1. Absent signal of the IC (Fam (Green) channel): retesting of the sample is required.
    - The PCR was inhibited.
        ⇒ Make sure that you use a recommended DNA extraction method and follow the manufacturer's instructions.
    - The reagents storage conditions didn't comply with the instructions.
        ⇒ Check the storage conditions
    - The PCR conditions didn't comply with the instructions.
        ⇒ Check the PCR conditions and for the IC detection select the fluorescence channel reported in the protocol.
    - No correct sample collection or preparation.
2. No signal on the Joe (Yellow)/Cy3/HEX and Fam (Green) channels with Positive Control.
    - The reagents storage conditions didn't comply with the instructions.
        ⇒ Check the storage conditions
    - The PCR conditions didn't comply with the instructions.
        ⇒ Check the temperature profile and select the fluorescence channel reported in the protocol.
    - Incorrect configuration of the PCR reaction:
        ⇒ Check the reagents preparation step.
3. Any signal with Negative Control.
    - Contamination during PCR preparation procedure. All sample results are invalid.
        ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
        ⇒ Pipette the Positive controls at the end.
        ⇒ Repeat the PCR preparation with the new set of reagents.

REFERENCES

- *High sensitivity of human leukocyte antigen-B*5701 as a marker of immunologically confirmed abacavir hypersensitivity in white and black patients*. Saag M et al. Clin Infect Dis 46: 1111 – 1118, 2008.
- *Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. Mallal S, Nolan D et al. Lancet. 2002 Mar 2;359(9308):722-3.*
- *HLA-B*5701 screening for hypersensitivity to abacavir. Mallal et al N Engl J Med. 2008 Feb 7;358(6):568-79.*
- *Value of the HLA-B*5701 allele to predict abacavir hypersensitivity in Spaniards. Rodríguez-Nóvoa S, García-Gascó P, Blanco F, González-Pardo G, Castellares C, Moreno V, Jiménez-Nácher I, González-Lahoz J, Soriano V. AIDS Res Hum Retroviruses. 2007 Nov;23(11):1374-6.*
- *Prospective HLA-B*5701 screening and abacavir hypersensitivity: a single centre experience.Waters LJ, Mandalia S, Gazzard B, Nelson M. AIDS. 2007 Nov 30;21(18):2533-4.*
- *Abacavir hypersensitivity reaction in primary HIV infection. Stekler J, Maenza J, Stevens C, Holte S, Malhotra U, McElrath MJ, Corey L, Collier AC. AIDS. 2006 Jun 12;20(9):1269-74.*
- *The pharmacogenetics of antiretroviral therapy. Phillips EJ. Curr Opin HIV AIDS. 2006 May;1(3):249-56.*

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
IVD	For *in Vitro* Diagnostic Use	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**C–**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

* iCycler iQ5™ is a registered trademark of Bio-Rad Laboratories
* Rotor-Gene™ is a registered trademark of Qiagen

Sacace Biotechnologies Srl
via Scalabrini, 44 – 22100 – Como – Italy Tel +390314892927
mail: ***info@sacace.com*** *web:* ***www.sacace.com***