HLA-B5701 RealFast™ Assay

REF 7-610 Σ 100 reactions

-20°C / 2-8°C CE IVD

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1. Intended Use

The HLA-B5701 RealFast™ Assay is a fast and accurate real-time PCR test for detection of the HLA-B5701 allele, a specific variant of the *human leukocyte antigen B (HLA-B)* gene that is strongly associated with abacavir hypersensitivity. The kit is designed for genetic risk stratification of HIV-infected patients prior to initiation of abacavir therapy. HLA-B5701 positive patients must be excluded from abacavir treatment. The qualitative assay discriminates the presence or absence of HLA-B5701 in a human genomic DNA extract. The kit does not interfere with the closely related HLA-B5702 and HLA-B5703 variants.
Reference sequence: HGVS: NG_023187.1

2. Introduction

Abacavir is a nucleoside analogue reverse-transcriptase inhibitor used to treat HIV-1 infection. Approximately 5% to 8% of Caucasians with HIV-1 infection who undergo a combination antiretroviral therapy containing abacavir develop a hypersensitivity reaction. Manifestations usually appear within the first 6 weeks after start of the treatment and include multisystem involvement resulting in skin rash, fever, constitutional, gastrointestinal tract or respiratory symptoms that become more severe with continued dosing. Immediate and permanent discontinuation of treatment leads to a rapid reversal of side effects. Conversely, re-challenge with abacavir after a hypersensitivity reaction can provoke potentially life-threatening conditions. Prospective employment of routine screening for HLA-B5701 before starting therapy significantly reduces the incidence of hypersensitivity reactions due to abacavir.

3. Kit Contents

RealFast™ 2x **Genotyping Mix**	1 vial	white cap	1000 µl
HLA-B5701 **Assay Mix**	1 vial	purple cap	550 µl
HLA-B5701 **Positive Control**	1 vial	green cap	75 µl

The kit contains reagents for 100 reactions in a final volume of 20 µl each.

The RealFast™ 2x Genotyping Mix comprises HotStart Taq DNA polymerase and dNTPs in an optimized buffer system.
The HLA-B5701 Assay Mix consists of gene-specific primers and dual-labeled hydrolysis probes for HLA-B5701 and a control gene.
A positive control for HLA-B5701 is supplied with the kit.

4. Storage and Stability

HLA-B5701 RealFast™ Assay is shipped on cooling blocks. On arrival, store the kit at -20°C. Alternatively, store at 2 to 8°C for short-term use within one month. The kit withstands up to 20 freeze/thaw cycles with no loss of activity. Avoid prolonged exposure to intense light. If stored correctly, the kit will retain full activity until the expiration date indicated on the label.

5. Product Description

5.1. Principle of the Test

The test is based on the fluorogenic 5' nuclease assay, also known as TaqMan® assay. Each reaction contains gene-specific primer pairs which amplify a 97 bp fragment of the HLA-B5701 gene and a 147 bp fragment of a control gene, the latter serving as PCR control. Further components are two dual-labeled, gene-specific hydrolysis probes which hybridize to the target sequence of the corresponding fragment. The proximity of the 5'-fluorescent reporter and 3'-quencher dye on intact probes prevents the reporter from fluorescing. During the extension phase of PCR the 5' – 3' exonuclease activity of the Taq DNA polymerase cleaves the 5'-fluorescent reporter from the hybridized probe. The physical separation of the fluorophore from the quencher dye generates a fluorescent signal in real-time, which is proportional to the accumulated PCR product.
In samples positive for HLA-B5701 both, the **FAM-labeled HLA-B5701** probe as well as the **HEX-labeled PCR control** probe bind to the appropriate gene fragment. A strong fluorescence signal is detected in the FAM channel (520nm) and in the HEX channel (556nm). In samples negative for HLA-B5701 only the HEX-labeled PCR control probe hybridizes to the complementary strand of the control gene fragment. A strong fluorescence signal is detected in the HEX channel and no or only a baseline signal in the FAM channel.

5.2. Real-time PCR Instrument Compatibility

The HLA-B5701 RealFast™ Assay is validated for use with the AB 7500 Fast instrument.
The kit is compatible with various common real-time PCR instruments capable of recording FAM and HEX fluorescence:

- ✓ AB 7500 Fast (Applied Biosystems®)
- ✓ AB StepOne™ (Applied Biosystems®)
- ✓ CFX96™ (Bio-Rad)
- ✓ LightCycler® 480 (Roche)
- ✓ Mx3005P (Agilent Technologies)
- ✓ Rotor-Gene® 6000 (Qiagen)

*» **Note:** RealFast™ Variant Detection QuickGuides for setting up and analyzing experiments on different types of instruments can be downloaded from www.viennalab.com.*

When using AB StepOne™, set passive reference dye to "ROX" ! «

The kit is supplied **without ROX**. For use with real-time PCR instruments requiring high ROX for normalization of data (e.g. Applied Biosystems® instruments: StepOne™, 7300, 7900/7900HT), add ROX at a final concentration of 1 µM to the 2x Genotyping Mix.

5.3. Assay Performance Specifications

Determination of **sensitivity** was performed on 64 alleles testing positive for the HLA-B5701 allele with a reverse SSO based reference kit. The HLA-B5701 RealFast™ Assay determined all 64 alleles as positive, which equaled a true positive rate of 100%.

Determination of **specificity** was performed on 66 alleles testing negative for the HLA-B5701 allele with a reverse SSO based reference kit. The HLA-B5701 RealFast™ Assay determined all 66 alleles as negative, which equaled a true negative rate of 100%.

Limit of detection: 0.2 ng genomic DNA (per reaction)
Recommended DNA concentration: 2 to 20 ng/µl genomic DNA

6. Materials Required but not Supplied

Real-time PCR instrument with FAM (520 nm) and HEX (556 nm) filters, instrument-compatible reaction vessels, disposable powder-free gloves, vortexer, mini-centrifuge for 2.0 ml tubes, tube racks, set of calibrated micropipettes (0.5 – 1000 µl), sterile tips with aerosol-barrier filter, molecular grade water, DNA extraction system, freezer, biohazard waste container.

7. Experimental Protocol

7.1. DNA Extraction

DNA extraction reagents are **not supplied** with the kit.
DNA isolated from various specimens (e.g. whole peripheral blood, dried blood spots, buccal swabs or saliva) can be used. Ensure extracted DNA is suitable for amplification in terms of concentration, purity and integrity.
For accurate genotype calling, the DNA amount per reaction should be within the range of 10 to 100 ng for all samples.

7.2. PCR Controls

Always include a **No Template Control** (NTC) in each experiment to confirm absence of potential contamination. It is advisable to run the NTC (use PCR-grade water instead of DNA) in duplicate.

Always include the HLA-B5701 **Positive Control** as positive reference signal for your unknown samples.

» ***Note:*** *The Positive Control is a potential source of contamination. Make sure to handle it carefully.* «

7.3. Preparation of HLA-B5701 RealFast™ Master Mix

Gently vortex and briefly centrifuge all solutions after thawing. Set up PCR at room temperature. Prepare sufficient **Master Mix** for all your reactions (N samples + positive control + negative controls) plus at least one additional reaction to compensate for pipetting inaccuracies:

Component	per reaction	e.g. 24+1 reactions
RealFast™ 2x Genotyping Mix	10 µl	250 µl
HLA-B5701 Assay Mix	5 µl	125 µl
Master Mix	**15 µl**	375 µl

Dispense **15 µl Master Mix** into each well. Add **5 µl** purified **DNA** or **Control** template to reach a final reaction volume of 20 µl.
To minimize risk of contamination, always pipette templates in the following order: first NTC, then samples, last positive control. Immediately close reaction vessels.

» ***Note:*** *Avoid creating bubbles in the final reaction mix and avoid touching the optical surface of the cap or sealing film without gloves. Both may interfere with fluorescence measurements. Centrifuge briefly if needed.* «

7.4. PCR Program

Program the real-time PCR instrument according to the manufacturer's instructions for quantitation experiments with two targets / reporter dyes. Place the samples into the thermal cycler and run the following program:

AB 7500 Fast, StepOne™, CFX96™, LightCycler® 480, Mx3005P and **other** Peltier heating block-based **instruments**:

Cycles	Temp	Time	Steps
1	95°C	3 min	Initial denaturation
40	95°C	15 sec	Denaturation
	60°C	1 min	Annealing/Extension – **Data acquisition** on FAM and HEX channel

Rotor-Gene® 6000:

Cycles	Temp	Time	Steps
1	95°C	3 min	Initial denaturation
40	95°C	15 sec	Denaturation
	36-well rotor: **56°C**	1 min	Annealing/Extension – **Data acquisition** on Green and Yellow channel
	72-well rotor: **60°C**		

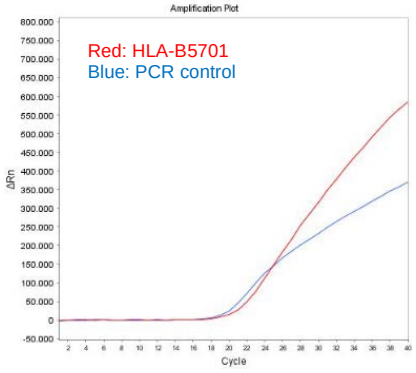
8. Data Analysis / Interpretation of Results

The presence or absence of the HLA-B5701 allele is defined by whether there is a signal in the **FAM channel** or not. Successful PCR can be verified by an amplification of the control gene detected in the **HEX channel** (PCR control). Thus, genomic DNA samples positive for HLA-B5701 as well as the HLA-B5701 Positive Control show an amplification in both, the HEX and FAM channel. HLA-B5701 negative samples show an amplification in the HEX channel only. Fluorescent levels and corresponding amplification curves are automatically displayed in amplification plots in the real-time PCR software.

Sample Type	Amplification in **FAM** channel (520 nm)	Amplification in **HEX** channel (556 nm)
HLA-B5701 positive	**YES**	**YES**
HLA-B5701 negative	NO	**YES**
HLA-B5701 Positive Control	**YES**	**YES**
NTC	NO	NO

Some instrument software may need manual threshold settings for accurate analysis.

Recommendations for Threshold Settings (C_q):
Set threshold value for the FAM and for the HEX channel just above the background fluorescent signal generated by the No Template Control (NTC).



Amplification plot: **HLA-B5701 positive** sample.

9. Warnings and Precautions

- For *in vitro* diagnostics use only.
- Always use disposable powder-free gloves and wear suitable lab coat when handling specimens and reagents.
- Perform reaction setup in an area separate from nucleic acid preparation and PCR product analysis.
- Use pipettes dedicated for PCR setup only, use aerosol-guarded pipette tips.
- Use instrument-compatible reaction vessels with optically clear caps or sealers.
- Do not mix reagents from different lots.
- Do not use expired kits or kit components.