BIOFORTUNA™
SIMPLY DIAGNOSTICS

Product Information Document

Product Number:	BF-10-11
Product Family:	SSPGo™
Product Name:	HLA-B*57:01 Identification Kit
Product Description:	8 reaction HLA-B*57:01 identification by SSP genotyping
Product Packaging:	8 reactions per test. Single sets of 8 HLA-B*57 reactions within individual foil pouch
Tests per Kit:	12
No template control:	Separate no template control (NTC)
Control amplification:	1400bp DRA control
Stability:	16 Months from manufacture. See pack details for date. Store between 4-30°C Once a foil pouch is opened use within 3 hours

Product specifications

BF-10-11 is designed to amplify all HLA-B*57 alleles in 8 PCR reactions and unequivocally identify B*57:01. It is recommended that patients requiring the drug Abacavir should be genotyped to identify B*57:01 as the presence of B*57:01 is a contraindication to the use of Abacavir.

HLA-B*57:01 background: Abacavir is an anti-retroviral drug used in treatment of HIV, however in sensitive individuals fever, skin rash, fatigue, gastrointestinal symptoms such as nausea, vomiting, diarrhea or abdominal pain and respiratory symptoms such as pharyngitis, dyspnea, or cough can develop.

A strong predictive association of *HLA-B*57:01* with this syndrome has been demonstrated (Mallal et al) and (Hetherington et al) with further evidence from recombinant haplotype mapping that the susceptibility locus/loci reside specifically with the 57.1 ancestral haplotype (AH), identified by the haplospecific alleles *HLA-B*57:01* and *C4A6* and the *HLA-DRB1*07:01, HLA-DQ3* combination. A haplotypic polymorphism within the tumor necrosis factor (*TNF*) promoter region (*TNF-238A*, associated with the 18.2 and 57.1 AHs) is also associated with abacavir hypersensitivity and may affect levels of TNF production, thereby influencing the severity of the syndrome. FDA has advised that people from at-risk ethnic groups be screened prior to drug-therapy (http://www.fda.gov/cder/drug/InfoSheets/HCP/abacavirHCP.htm FDA abacavir alert web access July 29, 2008).

Studies with CD8+ T cells responses to APC expressing single site mutations of B*57:01 indicate a critical role for residue 116 whereby the presence of Tyrosine at position 116 does not induce susceptibility to abacavir hypersensitivity, whereas serine 116 does. B*57:01 is serine 116 whilst B*57:02 an dB*57:03 are both tyrosine 116.

The frequency of B*57:01 varies in populations, but in general is about 8%. The ancestral B57.1 haplotype is about 5% in all populations, but tends to be higher in Caucasoid and Asian populations.

B*57:01, B*57:02 and B*57:03 are the only common subtypes of B*57. B*57:01 tends to be the dominant B*57 alleles in Caucasians and the Indian sub-continent. B*57:02 and B*57:03 are commonly found in African and Middle Eastern regions.

Version numbers: All Biofortuna kits have a version number. You must ensure the version number of the kit you are using matches the interpretation sheets and the version number in the software should you choose to use software. Version numbers change when there is a change in the kit that affects the results generated. This can occur (for example) if the primers in a kit change to accommodate a new allele or if an improved reaction has been created with a slightly different specificity to the one it replaced.

Version changes between kits: V1: Current Version

SSPGo General Description: SSPGo kits are unique freeze-dried assays where complete hot-start PCR reactions are pre-dispensed into 0.2ml PCR tubes. Each reaction in the kit contains a freeze-dried PCR solution consisting of a specific primer mix of allele and group-specific primers, a control primer pair for amplifying a fragment of the DRA1 gene and all the PCR ingredients including Taq polymerase, buffer, dNTPs, Magnesium Chloride, dyes and loading buffer. The hot start dNTPs are provided under license from Trilink. The PCR reaction is dispensed in 10µl volumes and just requires a 10µl DNA sample to rehydrate the primers prior to PCR.

Contents: Each assay is contained within a foil pouch also containing a disposable desiccant bag. The assay strip is sealed with caps that should be removed and discarded prior to adding DNA. The PCR vessels should contain 10µl of dry solid in the base of each well; this is the complete freeze-dried PCR reaction. For orientation the first reaction is always cresol red, which appears pale pink in the dry form. The remaining wells contain a blue dye which is the same colour wet or dry.

Interpretation: Paper interpretation sheets are available from www.biofortuna.com; to aid interpretation Biofortuna have created freely available software called Verdict™ which is available through the same link. Due to the complexity of the HLA system there will be occasions when certain combinations of alleles combine to produce an ambiguous result. It is therefore recommended that the software is used to help arrive at the correct interpretation. It is further recommended that you do not use these kits as the sole method of characterising HLA for clinical decisions.

Biofortuna SSPGo kits are designed to differentiate between alleles based on the first two digits. This is sometimes referred to as 'serological level'. The relationship between serological determinants and genotyping groups can be implied by this relationship, but for more information we direct you to the subjective listing found in 'The HLA dictionary 2008: a summary of HLA-A, -B, -C, -DRB1/3/4/5, and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR, and -DQ antigens' published by Tissue Antigens 2009: 73, 95–170.

Allele updates: All Biofortuna kits are updated on a regular basis with new alignments as they become available via IMGT HLA. Genotypes performed with kits using an earlier alignment can be retyped using updated kit information available from www.Biofortuna.com.

Primer information: The target sequence for the terminal six 3' bases of each primer are generally supplied. The forward primer information is shown as 5'-3' and the reverse primer is shown as 3'-5'. The primer location position is taken from the official alignments at http://www.ebi.ac.uk/imgt/hla/align.html.

No Template Control: Biofortuna's unique freeze-drying process greatly reduces the chance of PCR contamination because all you are adding is the DNA, i.e. no mixing of enzyme, buffers and DNA prior to adding to the primer mix. Therefore our single locus kits frequently do not contain a NTC well, which

means our kits have improved resolution due to the extra PCR reaction. No template control reactions suitable for Biofortuna kits are available (product number BF-40-02) and can be used separately for the genotyping kit. The NTC is designed to detect possible DNA contamination (either DNA or amplicon) in the diluent used for adding the DNA.

Validation: This is a CE marked product**.** All Biofortuna SSPGo kits are validated against at least 48 well characterised DNA samples.

Licenses: CleanAmp™ dNTPs are licensed from Trilink Biotechnologies Inc for use in Biofortuna SSPGo products. No license to perform PCR is required to use Biofortuna SSPGo kits.

References

Bunce et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens.* 1995 Nov;46(5):355-67.

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Hetherington S. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. *Lancet.* 2002 Mar 30;359(9312):1121-2.

Holdsworth et al. The HLA dictionary 2008: a summary of HLA-A, -B, -C, -DRB1/3/4/5, and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR, and -DQ antigens. *Tissue Antigens* 73, 95–170 (2009).

Mallal et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet.* 2002 Mar 2;359(9308):727-32.

Olerup & Zetterquist. HLA-DRB1*01 subtyping by allele-specific PCR amplification: a sensitive, specific and rapid technique. H. *Tissue Antigens*. 1991 May;37(5):197-204.

Revision history

This document is version 1. Dated 1-June-11