



BIOFORTUNA[™]
SIMPLY DIAGNOSTICS

Product Information Document

Product Number:	BF-10-27
Product Family:	SSPGo [™]
Product Name:	HLA-B*27 Identification Plus Kit
Product Description:	4 reaction HLA-B*27 genotyping by SSP
Product Packaging:	4 reactions per test. 2 sets of 4 HLA-B*27 reactions within individual foil pouch
Tests per Kit:	24
No template control:	Separate no template control (NTC)
Control amplification:	1000bp 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-10-27 is designed to amplify all HLA-B*27 alleles in four PCR-SSP reactions. The test gives unequivocal identification of HLA-B*27 and can be used to discriminate between B*27 alleles that may be negatively associated with disease such as B*27:06 and B*27:09.

Various publications have shown that B*27:06, B*27:07 negatively associated in Asian and Chinese populations whereas B*27:04 is positively associated with disease.

B*27:03 Negative association in African populations.

B*27:07 Is both positively and negatively associated in some reports.

HLA-B27 background: HLA-B27 associated with a spectrum of reactive arthritis diseases. The spondyloarthropathies (SpAs) are a family of related disorders that includes ankylosing spondylitis (AS), reactive arthritis (ReA; also known as Reiter syndrome [RS]), psoriatic arthritis (PsA), spondyloarthropathy associated with inflammatory bowel disease (IBD), undifferentiated spondyloarthropathy (USpA), and, possibly, Whipple disease and Behçet disease. Ankylosing spondylitis, which literally means "inflamed spine growing together," is the prototypical spondyloarthropathy.

Since the description of the close association of HLA-B27 with spondylarthropathies (AS), a significant proportion of the work in tissue-typing laboratories has been directed at assessment of HLA-B27 status. Requests for B27-typing by rheumatologists and other physicians have grown steadily despite frequent warnings that B27-status should rarely, if ever, be used as a diagnostic criterion for AS, as 8% of the (Caucasian) population is HLA-B27⁺ and only a small minority of these ever develop AS, instead the B27 test should ideally be used as a test of exclusion as it is very unlikely that a B27-negative patient has AS.

The HLA-B27 test may be ordered as part of a group of tests used to diagnose and evaluate conditions causing arthritis-like chronic joint pain, stiffness, and inflammation. This group of tests may include an RF (rheumatoid factor) with either an ESR (erythrocyte sedimentation rate) or a CRP (C-Reactive protein). HLA-

B27 is sometimes ordered to help evaluate someone with recurrent uveitis that is not caused by a recognizable disease process.

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 Information: 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™ software 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. Genotypings 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 product 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.

Bunce et al. Rapid HLA-DQB typing by eight polymerase chain reaction amplifications with sequence-specific primers (PCR-SSP). *Hum Immunol*.1993 Aug;37(4):201-6.

Bunce & Welsh. Molecular typing for the MHC with PCR-SSP. *Rev Immunogenet*. 1999;1(2):157-76.

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).

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