



Product Information Document

Product Number:	BF-10-04
Product Family:	SSPGo [™]
Product Name:	HLA-DQA1 Typing Kit
Product Description:	6 reaction HLA-DQA1 genotyping by SSP
Product Packaging:	6 HLA-DQA1 reactions within individual foil pouch
Tests per Kit:	10
No template control:	Separate no template control (NTC)
Control amplification:	700bp 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

Resolution: The SSPGo HLA-DQA1 typing kit is a medium resolution typing kit that contains pairs of 5'- and 3'-primers for resolving the HLA-DQA1 alleles into groups based on the first two digits of the allele name. Typing at the two digit level is sometimes referred to as low or medium level resolution, whereas defining the majority of alleles to a four digit level is often termed intermediate level. 'High resolution' is often used to describe the unique identification of an allele.

BF-10-04 is designed to identify the following groups of HLA-DQA1 alleles using 2-digit resolution: HLA-DQA1*01, *02, *03, *04, *05, *06

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: V2: 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 which also contains 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 has been published by Holdsworth et al (see references).

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.

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