



BIOFORTUNA[™]
SIMPLY DIAGNOSTICS

Product Information Document

Product Number:	BF-30-03
Product Family:	SSPGo [™]
Product Name:	Coeliac HLA Association Kit
Product Description:	Single multiplexed PCR-SSP reaction identifying DQA1 and DQB1 alleles associated with Coeliac disease (CD)
Product Packaging:	Strips of 1x8 reactions within individual foil pouch
Tests per Kit:	48
No template control:	Separate no template control.
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-30-03 is designed to amplify all DQA1*05 alleles, and the DQB1*02 and DQB1*03 alleles that are negative for the Asp57 polymorphism in a single multiplexed reaction. The DQB1*03 alleles containing non-Asp57 are alleles that can be defined as DQ8 (Holdsworth et al). Presence of DQA1*05 with DQB1*02 alleles or DQB1*03:02 alleles are associated with susceptibility to Coeliac Disease. Additional background information is given below.

Additional Information on Coeliac Disease Testing: Why use a genetic test for coeliac disease?

Coeliac disease develops because of intolerance to ingested wheat gluten or related proteins from rye and barley. Chronic inflammation in the small intestine results in villous atrophy and flattening of the mucosa. The primary HLA association in most coeliac disease patients is with DQ2 (DQA1*05/DQB1*02), and in a minority of patients with DQ8 (DQA1*0301/DQB1*0302) (See LM Sollid reference for review). Approximately 92-98% of patients with coeliac disease carry DQ2 while the remaining 2-8% of cases carry DQ8. Early diagnosis by antibody testing, DNA testing, and small bowel biopsy is critical. Treatment by elimination of gluten from the diet is essential for preventing future tissue damage and avoiding increased risk of other autoimmune disorders in affected individuals. Tissue trans-glutaminase antibody and anti-endomysial antibody test results can be equivocal depending upon diet adherence and stage of disease. However, coeliac disease DNA test results are always reliable and need only be performed once in a lifetime.

Genotyping HLA-DQA and DQB alleles is used as a test of exclusion to “rule out” CD, as it is unlikely that an individual with a negative genotyping test will develop CD in the future (Lui et al). There are several reasons for using the genetic test when evaluating an individual for CD. Primarily, the test can be used for individuals at familial risk of CD, where a negative test indicates the individual would not be required to have regular antibody screening for the remainder of their lives. For example, the children of an adult with CD could have the genetic test and the results would allow the family and physician to know which children need close monitoring.

The test can be used in individuals with CD-like symptoms to exclude the requirement for a biopsy in negative individuals. A positive genotyping test, however, does not diagnose the disease but increases the likelihood that it is present. The test can be used where there are contraindications in the use of the anti-endomysial antibody (anti-EMA) test, or where it is suspected that the anti-EMA test is false negative (Pearce et al).

UK NICE guidelines (<http://www.guideline.gov/syntheses/printView.aspx?id=16419>) on CD genotyping states: 'Approximately 40% of the general population in the United States have either the HLA class II heterodimer HLA-DQ2 or HLA-DQ8, which reflects the presence of the DQ alleles DQA1*05 and DQB1*02 (DQ2) or DQA1*03 and DQB1*0302 (DQ8). However, almost all patients with CD have either DQ2 (~95% of patients with CD) or DQ8 (~5% of patients with CD). A very small number of patients with CD have been noted to have only DQA1*05 or DQB1*02, the latter usually being associated with HLA-DR7 heterozygosity or homozygosity.

Because virtually all patients with CD have the CD-associated alleles mentioned previously at the DQA1 and DQB1 loci, the absence of these alleles provides a negative predictive value for the disease of close to 100% (i.e., if individuals lack the relevant disease-associated alleles, CD is virtually excluded). HLA testing for the relevant DQ alleles can be a useful adjunct in an exclusionary sense when the diagnosis based on other tests is not clear. When using HLA testing in the context of disease susceptibility in families, one must have the resources available to provide genetic counselling.

What does the Biofortuna Coeliac HLA Association Kit identify?

The Biofortuna SSPGo Coeliac HLA Association Kit is a multiplexed single PCR test for the HLA-DQ genes and polymorphisms required for the development of coeliac disease.

The test identifies the presence of the CD-associated allele groups HLA-DQB1*02, DQB1*03:02 and DQA1*05. The HLA-DQB1*02 and HLA-DQB1*03 alleles are identified in the context of absence of aspartic acid on the DQ beta chain at position 57 (Asp57) an absence that is shared by most DQB1*02 alleles and a subset of DQB1*03 (DQB1*03:02 group) alleles known as 'DQ8'. Absence of Asp57 on the DQ beta chain is required for the development of coeliac disease (Hovhannisyan et al, and e-publication <http://accessscience.com/content/Celiac-disease/YB110031>).

Interpretation of the Biofortuna Coeliac Genotyping Test

The Biofortuna Coeliac Genotyping Test is a multiplexed PCR that identifies all of the relevant alleles in Coeliac disease susceptibility. The identification of the size of the amplicons is required for interpretation of the test and it is recommended that a known positive DNA is used for every batch of test performed and/or that a suitable 100bp ladder is run alongside any sample for testing. The test will produce the following results:

	Control	DQ8 DQB1*03:02 group	DQA1*05 group	DQ2 DQB1*02 group			
Amplicon Sizes	1000bp	135bp	230bp	410bp	Result	Asp57	CD susceptibility*
Possible results	+	+	+	+	DQB1*02 grp, DQB1*03:02 grp & DQA1*05 group	Asp57 -ve	High risk
	+	-	+	+	DQB1*02 grp, & DQA1*05 group	Asp57 -ve	High risk
	+	+	+	-	DQB1*03:02 grp & DQA1*05 group	Asp57 -ve	Moderate risk
	+	+	-	-	DQB1*03:02 group	Asp57 -ve	Moderate risk
	+	+	-	+	DQB1*02 grp & DQB1*03:02 group	Asp57 -ve	Moderate risk
	+	-	-	+	DQB1*02 group	Asp57 -ve	No risk
	+	-	+	-	DQA1*05 group	Asp57+ve	No risk
	+	-	-	-	Negative	Asp57+ve	No risk

The CD susceptibility information is developed from table 1 in Dubois and van Heel's review.

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 First version: no previous version

SSPGo General Description: Biofortuna SSPGo kits are unique freeze-dried assays where complete hot-start PCR reactions are pre-dispensed into 0.2 ml 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 1000bp 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 Biotechnologies Inc. 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 plate is sealed with a foil thermally bonded sheet that should be removed 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™. For version 1 of this kit there is no Verdict file as the interpretation is simple to do using the interpretation tables.

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 an 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: 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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Dubois & van Heel. Translational Mini-Review Series on the Immunogenetics of Gut Disease: Immunogenetics of coeliac disease. *Clinical and Experimental Immunology*, 153: 162–173 (2008).

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Revision history.

This document is version 2. Dated 23-August-11

Correction of Asp57 status in table.