



# Instructions for Use for Biofortuna SSPGo™ HLA Typing Kits

**Version 4**

**June 2011**

## 1. Intended Use

Biofortuna HLA SSPGo Kits are qualitative DNA-based kits for determining HLA alleles in either 'high resolution' kits, or group-specific amplification of alleles in 'medium level' resolution kits. The common definition of medium level resolution is where the majority of results are clearly defined at the two digit level; e.g. DQB1\*02, DQB1\*05. High resolution is generally defined as the majority of alleles identified are defined at the four-digit level such as DQB1\*02:01, DQB1\*05:01. This is an *in vitro* diagnostic product intended for use by trained personnel only.

## 2. Introduction

HLA molecules play a key role in immunity and recognition of self versus non-self, consequently HLA genotyping and HLA matching is mandatory prior to most forms of transplantation. As HLA antigens restrict the specificity of T-cell mediated immune responses HLA genotyping is a useful investigative tool in any immune disorder or any immune response to pathogens, vaccines or medical treatment. HLA genotyping can also be used to support disease diagnosis where certain HLA alleles have been shown to be significantly associated with disease states.

Most HLA genes are highly polymorphic and generally DNA genotyping is required for accurate determination of HLA antigens. PCR genotyping using Sequence-Specific Primers (SSP)<sup>1</sup>, is a rapid method of HLA genotyping, particularly suitable for situations where medium level resolution is required. Biofortuna SSPGo kits all feature complete dried reactions, including polymerase, so that all the user has to do prior to PCR is add DNA.

Every effort is made to keep the kits updated with new IMGT HLA alignment releases. Kit updates are available from [www.biofortuna.com](http://www.biofortuna.com).

## 3. Test Description

PCR SSP is based on the principle that only primers with completely matched 3' terminals to a target sequence will amplify. Mismatched primers do not yield positive amplification products<sup>2</sup>. An internal control primer pair, which amplifies a conserved region of a housekeeping gene, is included in every PCR reaction mix; the internal control primer pair is an indicator of the integrity of the PCR reaction. SSP genotyping generally uses multiple reactions that when analysed together indicate the genotype. Visualisation of the amplified products can be achieved using agarose gel electrophoresis systems which separate the DNA fragments by size.

## 4. Kit Contents

- 10-40x Polypropylene PCR trays or strips consisting of between 1 and 96 PCR vessels (kit dependant), each vessel containing 10µl pre-dispensed freeze dried primers, polymerase, dNTPs\* and buffer. Each test or strips are individually packed within a foil pouch.
- 1x Instructions for use.
- Interpretation tables, MSDS & Certificate of analysis can be downloaded from the Biofortuna website [www.biofortuna.com](http://www.biofortuna.com). If you are unable to download from the website please contact your local distributor.

\*CleanAmp™ dNTPs are licensed from Trilink Biotechnologies Inc for use in Biofortuna SSPGo products.

## 5. Reagents and Equipment Not Supplied

- Appropriate pipettors and sterile tips e.g. P10 pipettor with 10µl filter tips.
- DNA isolation kit/equipment.
- UV spectrophotometer.
- Polypropylene tubes.
- Sterile molecular grade water.
- PCR sealing sheets or caps. (Strip caps are provided for kits with strip tubes)
- 96 well thermal cycler with heated lid. PCR plates and tubes used in Biofortuna kits have been validated for use with the majority of thermocyclers on the market, including MJ Research PTC-100, PTC-200, Hybaid MBS and Techne TC-512 thermal cyclers. Different models may require further validation by the user.
- Gel electrophoresis reagents (agarose, 0.5x TBE, 1000bp DNA molecular weight marker, 10mg/ml Ethidium Bromide).
- Gel electrophoresis equipment (gel tanks, power supply, gel documentation system with UV transilluminator).

## 6. Safety and Warnings

- For *in vitro* diagnostic use.
- Tests should only be carried out by appropriately trained personnel.
- All typing results should be verified by qualified personnel and if used for a clinical decision the results should be confirmed using another typing method.
- Handle all reagents in accordance with Good Laboratory Practice.
- Keep pre- and post-PCR areas separate. Do not bring any post-PCR materials back to the pre-PCR area.
- **Biohazard Warning:** Treat all blood products as potentially infectious.
- **Biohazard Warning:** Ethidium Bromide is a potential carcinogen. If used, always wear gloves, a laboratory coat and protective eye glasses.
- **Biohazard Warning:** Take care when using UV sources - always wear gloves, a laboratory coat and protective eye glasses. Never view the UV light source directly.
- Material Safety Data Sheets are available from [www.biofortuna.com](http://www.biofortuna.com).

## 7. Storage and Stability

Biofortuna SSPGo kits should be stored at 4-30°C. Once PCR vessels are removed from the foil pouches the reagents should be re-hydrated with DNA within 3 hours. Refer to packaging for expiration date. Do not use products after the printed date.

Do not use kits if the foil pouch is ripped or perforated.

Ensure PCR vessels are sealed tightly after adding DNA as this may lead to evaporation during PCR amplification. Pay particular attention to edges and corners.

## 8. Directions for Use

### DNA Sample Requirements

Each reaction in the test is optimised to utilise between 50 - 100ng of DNA, but it is critical that each reaction should be re-hydrated with exactly 10µl of liquid. Therefore, the test can be performed with 10µl of DNA at 5-10ng/µl, or a volume of water can be added first to allow addition of DNA at a higher concentration, e.g. adding 9µl of water followed by 1µl of DNA at 50-100ng/µl. As heparin may inhibit PCR it is recommended that DNA should not be extracted from heparinised blood samples. The OD<sub>260/280</sub> of the DNA sample should fall between 1.6 and 2 as measured by UV spectrophotometry.

**Pre-PCR Directions**

- i. Remove an SSPGo tray or strip from a sealed pouch.
- ii. Note the lot number, product number and version of the assay(s).
- iii. Note that the first reaction of each test locus is always red in colour to the rest of the kit.
- iv. Some PCR trays contain a purple coloured integral ‘no template control’ reaction in the last well of the tray.
- v. Using sterile equipment pipette 10µl DNA solution into each reaction of the tray or strip. See note, in Section 8 on DNA Sample Requirements. If the tray contains a purple coloured integral ‘no template control’ then pipette 10µl of sample diluent (without DNA) to it. See note on No Template Control in Section 8.
- vi. Ensure the DNA contacts the dry reagents in each reaction prior to thermal cycling. A brief centrifugation step may be used to ensure all DNA solution has contacted the dry reagents.
- vii. Seal the reactions with a sealing sheet or PCR tube caps. Ensure the seal is as tight as possible to prevent evaporation. Pay particular attention to edges and corners.
- viii. Place tray or strips directly into the thermal cycler. Ensure the vessels are fully inserted into the block and the lid is fully compressed. Failure to do so can lead to individual PCR failure. It may be necessary with some models of PCR machines to use compression pads or blocks to get effective compression of the product in the block.
- ix. Run PCR program (refer to PCR Parameters).

RE-SUSPENSION NOTE: Ensure the PCR mixes are re-suspended with the DNA sample within 3 hours of removing the tray from the foil pouch.

NO TEMPLATE CONTROL NOTE: Some kits include a no template control (NTC) as the final reaction on the plate. This reaction contains a purple dye to distinguish it from the rest of the reactions, and its presence in a kit is also noted on the accompanying interpretation tables. The NTC is designed to detect PCR contamination, or genomic DNA contamination from Biofortuna SSPGo kits that may be present in the water used to re-suspend your DNA. If PCR contamination is present variable size amplicon(s) will be observed, if the contamination is genomic DNA an 187bp amplicon will be observed.

PCR PLATE/STRIP HEIGHT PROFILE NOTE: It is recommended that the height profile of plates and strips are equivalent when placed in the same PCR machine. Different height profiles can cause poor contact with the PCR machines heated lid. This may result in poor or failed PCR amplification.

**PCR Parameters**

The following PCR parameters should be used. Ensure ramp speeds of at least 1°C per second and enable the heated lid. Please refer to the thermocycler manufacturer’s user manual for full instructions for use. Thermalcyclers should be calibrated according to the American Society of Histocompatibility and Immunogenetic (ASHI) or European Federation of Immunogenetics (EFI) accreditation rules.

Denature	94°C	5 minutes		
Denature	96°C	15 seconds	←	10 cycles
Anneal	66°C	50 seconds		
Extend	72°C	30 seconds		
Denature	96°C	15 seconds	←	20 cycles
Anneal	64°C	50 seconds		
Extend	72°C	30 seconds		
HOLD	15°C			

## Gel Electrophoresis

These instructions apply to horizontal agarose gel electrophoresis: Prepare a 2% agarose gel in 0.5x TBE buffer. When the gel is cooled to about 60°C add ethidium bromide to a final concentration of 0.5µg/ml. Cast gel and insert microtitre format combs (e.g. 12x8 wells with 9mm spacing). Once set, remove the combs and cover gel in 0.5x TBE buffer. Transfer a minimum of 5µl and a maximum of 10µl from each tray or strip reaction to the corresponding well on the gel, noting the position of each reaction. A 100bp ladder can be useful to aid size determination. Run gel for 20 minutes at 10V/cm.

Refer to your electrophoresis system manufacturer's instructions for use for specific equipment details. Gels should be imaged using a UV gel documentation system with UV transilluminator.

## 9. Interpretation

SSPGo kits are designed so the results can be determined manually using interpretation tables available from [www.biofortuna.com](http://www.biofortuna.com). If you have trouble accessing the website please contact your local distributor.

Affix the gel photograph to the corresponding interpretation form by matching the kit and version numbers. Examine the gel image. Each reaction should contain a positive control band. Refer to the interpretation tables as this may be a different size in different SSPGo products. Internal control bands might appear much weaker when allele specific bands are present. If an allele specific band is present but a control band is not, this should still be considered a positive result. Ignore any bands less than 70bp as these are unincorporated primers.

Determine the positive reactions. Positive reactions are indicated by bands of the expected size, as stated in the interpretation tables. Be aware that there may be more than one product size in a given reaction – these are multiplexed reactions and are noted on the interpretation tables.

Compare positive reactions with the interpretation tables. A positive result in a reaction indicates the presence of at least one of the alleles listed against it on the interpretation table. Any given allele may be amplified in several tubes – if the allele is present there should be a positive reaction in all of the relevant reactions.

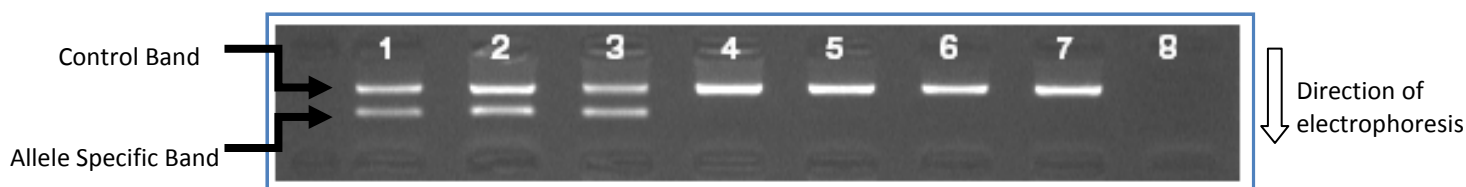


Figure 1: Examples of positive reactions, indicated by the presence of allele specific bands and control bands (reactions 1-3); negative reactions, indicated by the presence of control bands but absence of allele specific bands (reactions 4-7); and a failed reaction, indicated by the absence of any bands (reaction 8).

**Ensure the kit version is matched correctly with the version on the interpretation table.**

## 10. Quality Assurance and Control

Each SSPGo batch is checked for quality before any product leaves Biofortuna. Samples of each kit batch are checked against a defined panel of human DNA samples to ensure correct performance. Each reaction has been validated against at least 48 well characterised cell line DNA samples. Biofortuna recommend that any laboratory should internally validate any new typing products before use on clinical samples. Only fully trained and qualified personnel should perform diagnostic typing, and results should be cross checked by another trained member of staff.

## 11. References

- 1) Bunce M et al Tissue Antigens. 1995 Nov;46(5):355-67.
- 2) Saiki RK et al. Nature. 1986 Nov 13-19;324(6093):163-6.

## 12. SSPGo Troubleshooting Guide

Problem	Probable Cause	Remedy
<b>No amplification in any reaction</b>	Incorrect concentration of DNA used	Measure the DNA quantity and ensure 50 - 100ng of DNA in total is added in a volume of 10µl, per reaction.
	PCR inhibitors present in DNA sample	Avoid heparinised blood, or if unavoidable, extract DNA from washed intact lymphocytes so that heparin does not contact the DNA.  Protocols using heparinase to remove the heparin from DNA samples have been described.  Re-extract DNA. See manufacturer's guidelines for DNA extraction kit use.
	Poor quality DNA sample used	Measure the DNA quality. The A260/A280 ratio should be 1.6 – 2.0 by UV spectrophotometry.  Ensure that the DNA is fully re-suspended in solution before use.
	Reagents not fully re-suspended	Ensure pellets are fully re-hydrated on addition of DNA. If necessary, briefly centrifuge the plate.  Ensure 10µl of DNA solution is used per reaction.
	Thermal cycler not set up correctly	Ensure that the PCR program has been entered correctly, according to the instructions for use.  Ensure that the thermal cycler's heated lid is engaged and sufficiently tightened.  Refer to the thermal cycler's instructions for use for further guidance.
	Electrophoresis problems	Ensure there is power to the electrophoresis tank – check the power pack and clean the electrodes.  Run the gel in 0.5X TBE buffer.  Ensure 0.5µg/ml of fresh ethidium bromide is used. Check that there is sufficient UV illumination when imaging gels.  Refer to the gel tank and power pack manufacturer's instructions for further guidance.
	Plates not sealed correctly.	Insufficiently sealed plates can lead to evaporation during PCR. Biofortuna supplies recommended sealing sheets (product number BF-40-11).  Ensure there is an adequate seal across all the wells. Pay particular attention to the wells close to the edges of the PCR tray or strip.







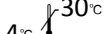

Problem	Probable Cause	Remedy
<b>Random drop-outs of control and/or allele specific amplicons</b>	Gel errors	<p>Ensure that all of the wells have been loaded onto the gel in the correct order, and the same volume of PCR reaction was added to each one.</p> <p>Calibrate pipettors as described by the manufacturer's instructions.</p> <p>Check that the wells are properly formed in the gel. Take care when removing combs as it is possible to tear the bottom of the wells.</p> <p>Ensure that the agarose is fully dissolved before casting the gel.</p> <p>Ensure that the gel is not run too long, as smaller amplicons may run off the end.</p> <p>Ensure the gel has ran long enough to allow bands to separate.</p> <p>Use fresh ethidium bromide solution.</p>
	Thermal cycler problems	<p>Failures, particularly around the edge of the assay may be due to not tightening the lid sufficiently. This can lead to evaporation and condensation of the PCR reaction half way up the PCR vessel and can lead to PCR failure.</p> <p>Be sure to follow the manufacturer's guidance for the maintenance and calibration of your thermal cycler.</p> <p>Check the PCR parameters are correct, according to the instructions for use.</p>
	Evaporation problems	<p>Ensure there is an adequate seal across all the wells. Pay particular attention to the wells close to the edges of the PCR tray or strips.</p> <p>Ensure the heated lid is enabled and sufficient compression is applied via the lid. Biofortuna supplies recommended sealing sheets (product number BF-40-11).</p>
	Sporadic failure due to DNA problems	<p>No DNA present: Ensure DNA is present in all wells.</p> <p>Wrong volume: Ensure 10µl of DNA solution is added to each reaction.</p> <p>Too much DNA added: Concentration of above 200ng may cause PCR failure.</p> <p>Contaminants in DNA may lead to sporadic or widespread failure to amplify.</p>

Problem	Probable Cause	Remedy
<b>Smeary gel image</b>	DNA	Check the concentration and purity of the DNA. Adding too much DNA to the PCR reactions can result in smeary gel images.
<b>Weak amplification</b>	DNA concentration problem	Check the DNA concentration is neither too high nor low. Aim for 100ng of DNA per reaction, in 10µl.
	Thermal cycler problems	Be sure to follow the manufacturer's guidance for the maintenance and calibration of your thermal cycler.  Check the PCR parameters are correct, according to the instructions for use.
	Gel errors	Ensure that the same volume of reaction was added to each well, between 5µl and 10µl.  Calibrate pipettors as described by the manufacturer's instructions.  Use fresh ethidium bromide solution.
<b>Non-specific amplification</b>	DNA concentration problem	Check the DNA concentration is neither too high nor low. Aim for between 50 - 100ng of DNA per reaction, in 10µl.
	Reactions loaded in the incorrect order	Check alignment of PCR and gel lanes.  Prevent physical overflow from adjacent wells in electrophoresis by not overloading and making sure gel is set before removing combs.
	New allele identified	Previously un-sequenced alleles may be present with a new amplification pattern. If using old interpretation sheets then download a more current alignment update from <a href="http://www.biofortuna.com">www.biofortuna.com</a> . If this does not accommodate the new pattern you should check by using a different Biofortuna kit, or attempt to identify the sequence by sequence-based typing. Alternatively contact Biofortuna technical service for more help and advice.
<b>Amplification pattern is not interpretable</b>	Incorrect interpretation of an artefact as a specific band	Check the version-specific Interpretation Tables for correct band size.  Check if all specific amplifications are correct in size or if an artefact (carry-over, primer dimer) has been misinterpreted as an amplification.
	Reactions loaded in the incorrect order	Check alignment of PCR and gel lanes.
	Individual PCR failure	Check all internal positive controls are present. Re-interpret without any missing reactions.
	Small amplicons missing	Electrophoresed too far, small amplicons have run off the end of the gel, or past the ethidium bromide front, or are dispersed by entering preceding gel well. Use electrophoresis conditions suitable for your gel system.

Problem	Probable Cause	Remedy
	New allele identified in sample	New alleles may occasionally be discovered that may give rise to an amplification pattern that does not correspond to an existing allele(s). Biofortuna technical service would be happy to help you confirm any such new allele by additional methods at our disposal. Please contact your local distributor.

13. Notes

## 14. Guide to Symbols Used

	Number of Tests
	EC Representative
	Consult Instructions for Use
	Site of Manufacture
	In Vitro Diagnostic
	Expiry Date
	Storage Temperature
	Lot Number

## 15. Manufacturer Contact Details

Biofortuna Ltd  
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 E: [info@biofortuna.com](mailto:info@biofortuna.com)  
 W: [www.biofortuna.com](http://www.biofortuna.com)



## 16. Translations

Française:	Traductions disponibles
Deutsch:	Übersetzungen verfügbar
Español:	Traducciones disponibles
Italiano:	Traduzioni disponibili
České:	Překlady k dispozici
Danske:	Tilgængelige oversættelser
Ελληνες:	διαθέσιμες μεταφράσεις
Magyar:	Fordítások
Norske:	Oversettelser tilgjengelig
Polska:	Dostępne tłumaczenia
Português:	Traduções disponíveis
Россия:	Переводы доступны
Slovenskému:	Preklady k dispozícii
Türk:	Çeviriler mevcut
Svenska:	Översättningar tillgängliga

[www.biofortuna.com](http://www.biofortuna.com)