Instructions for Use Biofortuna SSPGo™ HLA Wipe Test BF-40-01

USA version 1, May 2014

1. Intended Use

The Biofortuna SSPGo HLA Wipe Test is intended to be used as a Quality Control test to monitor laboratory areas and equipment for PCR amplicon contamination that may be generated by the use of Biofortuna SSPGo products.

2. Introduction

PCR is a sensitive technique that is susceptible to contamination with DNA amplicon from a previous PCR. Contamination can lead to false positive amplification in subsequent PCRs, which can lead to incorrect genotyping. PCR amplicon can contaminate reagents and samples, as well as laboratory equipment such as pipettes. Reagents and equipment should be monitored regularly for signs of contamination.

3. Test Description

Each SSPGo HLA Wipe Test consists of a strip of eight PCR wells containing freeze dried PCR buffer, polymerase and primers specific for the HLA-DRA gene and will produce an 187bp amplicon from human genomic DNA. Since all Biofortuna kits utilise a DRA amplicon as an internal control, any contamination from use of Biofortuna SSPGo kits will have amplification of the DRA gene and will be detected by the wipe test primers.

Each strip of eight wells tests up to three wipe test zones for contamination. Each strip has integral positive and negative control reactions, as well as inhibition control reactions for each wipe test zones. To test a zone for contamination it is first wiped with a swab which is then soaked in water. This water is used as a template in the wipe test, and as a 50:50 mixture with genomic DNA as a PCR inhibition test.

It is recommended to test for contamination on a regular basis. Typical zones to be tested include DNA preparation area, PCR setup area and post-amplification area. Typical items to be tested include work benches, pipettes, centrifuges, refrigerator and freezer handles, door knobs and racks. Typical solutions to be tested include DNA preparation buffers and DNA diluents. Multi-use shared reagents such as PCR buffers and *Taq* polymerase are particularly susceptible to contamination but these do not affect Biofortuna kits since Biofortuna products are complete and only require the addition of DNA.

4. Kit Contents

- 12 strips of 8 PCR wells, each containing pre-dispensed freeze dried primers, polymerase, dNTPs* and buffer. Each foil packed strip is intended for testing three zones for contamination. The eight reaction strip format is shown below.

Reaction	Dye	Use
1	Red	Positive Control: DNA
2	Purple	Negative Control: water used to wet swab
3	Blue	Zone 1 Inhibition Test: 50% DNA, 50% wipe water
4	Purple	Zone 1 Wipe Test
5	Blue	Zone 2 Inhibition Test: 50% DNA, 50% wipe water
6	Purple	Zone 2 Wipe Test
7	Blue	Zone 3 Inhibition Test: 50% DNA, 50% wipe water
8	Purple	Zone 3 Wipe Test

- 36 sterile swabs
- 12x8 PCR caps
- 1x instructions for use and one Certificate of Analysis
- The MSDS can be downloaded from the Biofortuna website 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 calibrated pipettors and sterile tips e.g. P10 pipettor with 10µl filter tips
- DNA isolation kit/equipment
- UV spectrophotometer suitable for the measurement of DNA concentration and purity
- Polypropylene tubes
- Sterile molecular grade water
- A thermal cycler with the following specifications should be used:
  - 96 well thermal cycler with heated lid with a temperature of 104°C for oil-free operation
  - Ramp rate of 1.0°C/sec.
  - Temperature range of 4.0°C to 99.9°C
  - Temperature accuracy of ±0.25°C for the range of 35°C to 99.9°C
  - Temperature calibration traceable to a reference standard
  - Program the thermal cycler using the PCR Cycling Parameters in Section 8 below.

  Note: For specific thermal cycler information refer to the manufacturer's user manual. Thermal cycler should be calibrated according to ASHI (American Society of Histocompatibility and Immunogenetics) or EFI (European Federation of Immunogenetics) accreditation rules.
- 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)

Note: any change in the specified conditions, such as thermal cycler ramp rates, may affect the test results.

6. Safety and Warnings

- Tests should only be carried out by appropriately trained personnel.
- 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.

7. Storage and Stability

Biofortuna SSPGo HLA Typing kits should be stored at 2 – 28°C (36 - 82°F) Once PCR strips or plates are removed from the foil pouches the reagents should be re-hydrated with test sample promptly. See Note (1) and Note (2) for additional information.

Use only the sealing sheets or caps provided. Ensure PCR wells are sealed tightly after adding DNA as omitting this may lead to evaporation during PCR amplification. Pay particular attention to edges and corners. Once you have removed the original caps from the 8 well strips, **DO NOT re-use**. Only use the additional spare caps provided to carry out the PCR reaction.

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.

Note (1): If necessary, the out-of-pouch non-hydrated PCR plates and strips may be held for up to 3 hours prior to addition of sample at a temperature of up to 20°C and humidity of no more than 60%.

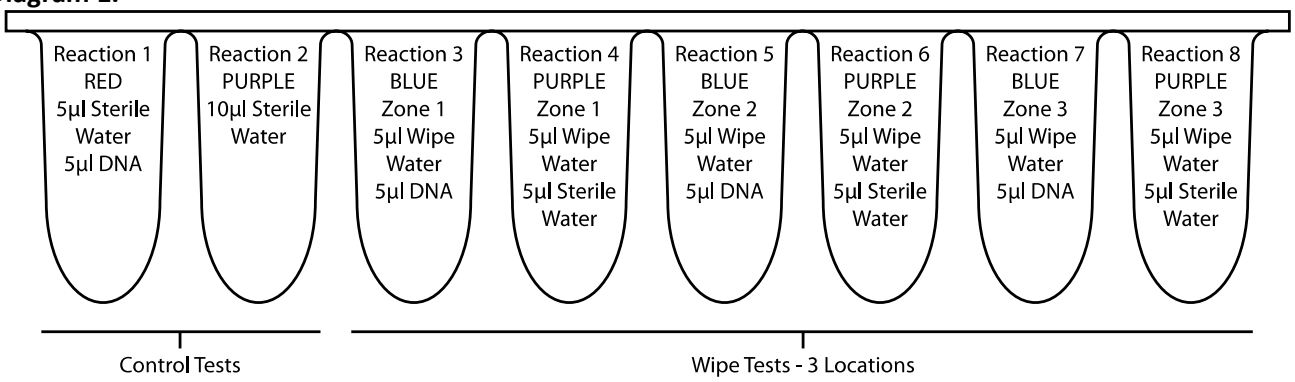
Note (2): Once hydrated with sample, PCR strips and plates from freshly opened pouches can be stored for up to 24 hours at 2–8°C before the PCR step, provided that the wells are well sealed to avoid evaporation.

8. Directions for Use

Note: A frequent source of contamination is PCR pipettes and it is advisable to swab the tip of the pipette and if possible inside the barrel. It is also recommended that all contamination tests are performed with a pipette that has been tested and shown to be negative. If contamination is discovered follow your laboratory guidelines to eradicate contamination and retest zones.

1. In a DNA free location, label a sterile DNase-free polypropylene 2ml tube for each of the wipe test zones to be tested. Label an extra tube 'Negative Control'
2. Using a known contamination free pipette, add 500µl sterile, molecular grade distilled water to each of the 2ml tubes.
3. Wet a provided sterile plastic applicator swab in each wipe test tube.
4. Wipe the zone to be tested with the moistened applicator.
5. Snap or cut off the plastic stem of the applicator and place in the original 2ml tube of water.
6. Vortex briefly.
7. Remove and discard the swab with sterile forceps.
8. Centrifuge, 1 minute, 10,000 to 13,000 rpm in a micro-centrifuge to remove particulate matter.
9. Open the test foil pouch[1]. All tests are to be rehydrated with a total of 10µl of liquid as shown in diagram 1.
10. **Negative control:** Add **10µl** sterile molecular grade water to the negative control reaction **2** (purple).
11. Add **5µl** of the sterile water to reactions **1, 4, 6, 8.**
12. **Positive control**: Add **5µl** of 10ng/µl human genomic DNA[2] to reaction **1** (red reaction), and reactions **3, 5 & 7** (blue).
13. **Wipe test reactions:** Add **5µl** of centrifuged liquid (wipe water) from wipe zone 1 to reactions **3 & 4**, from wipe zone 2 to reactions **5 & 6**, and from wipe zone 3 to reactions **7 & 8**.
14. All reactions now have 10µl rehydrated volume as shown in diagram 1. Cap the reactions with the provided caps and proceed with the usual SSPGo PCR parameters as shown below.

Diagram 1.



RE-HYDRATION NOTE: Once PCR wells are removed from the foil pouches the reagents should be re-hydrated with DNA promptly. See Note (1) and Note (2) for additional information.

[1] If necessary, the out-of-pouch non-hydrated PCR plates and strips may be held for up to 3 hours prior to addition of DNA at a temperature of up to 20°C and humidity of no more than 60%. Once hydrated with DNA, PCR strips and plates from freshly opened pouches can be stored for up to 24 hours at 2–8°C before the PCR step.

[2] Ensure the final DNA sample does not contain more than 2.5mM Tris/0.25mM EDTA. Only use DNA extracted from citrate and EDTA collected samples. As heparin may inhibit PCR it is recommended that DNA should not be extracted from heparinised blood samples. Hemoglobin has been shown to interfere with SSPGo HLA kits when present in DNA samples at greater than 1 mg/dL.

DNA can be extracted using traditional extraction methods. Ensure that the $OD_{260/280}$ of the DNA sample falls between 1.66 and 1.94 as measured by UV spectrophotometry.

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 1°C per second and enable the heated lid. Please refer to the thermal cycler manufacturer's user manual for full instructions for use. Thermal cyclers 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	
Anneal	66°C	50 seconds	10 cycles
Extend	72°C	30 seconds	
Denature	96°C	15 seconds	
Anneal	64°C	50 seconds	20 cycles
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

Test results for swabbed zones are only valid if the positive control is positive and the negative control is negative. Tests for swabbed zones are only valid if the corresponding inhibition control test is positive.

A 187bp amplicon should be observed if there is PCR contamination or DNA contamination present. Any smears or bands of different sizes may also indicate PCR contamination, but primer-dimer and other primer extension artefacts of less than 100bp should be ignored.

Rx	Dye	Use	Result	Conclusion	Action
1	Red	Positive control	187bp +ve	Test valid. DNA suitable. PCR effective	
			No amplification	Test invalid	Repeat entire assay with different DNA control
2	Purple	Negative Control	187bp +ve	Test invalid. Water and/or pipette contaminated	Test with different water and pipette
			No amplification	Test valid. Water not contaminated	
3, 5, 7	Blue	Inhibition Test	187bp +ve	No inhibitors present	
			No amplification	Potential inhibitors present	Clean area with sterile water, repeat
4, 6, 8	Purple	Wipe Test	187bp +ve	Contamination is present	Isolate and clean pipette used. Clean contaminated area with solution designed to remove DNA. Repeat test.
			No amplification	Wipe test negative. No contamination	

10. Quality Assurance and Control

Assay testing: PCR amplicon from a Biofortuna Kit was allowed to dry on a solid surface. The wipe test was performed on the amplicon undiluted and then in dilutions from $1x10^{1}$ to $1x10^{15}$. The amplicon was detected in dilutions up to and including $1x10^{15}$.

Genomic DNA was allowed to dry on a solid surface. The wipe test was performed on gDNA at 100ng/µl and then in dilutions from $1x10^{1}$ to $1x10^{15}$. The DNA was detected using gDNA with concentrations ranging from 0.1ng/µl to 100ng/µl.

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. HLA Wipe Test Sample record sheet

Sample Record Sheet It is recommended that this sample record sheet is photocopied prior to use as the Wipe Test Kit has sufficient tests for 36 zones (3 wipe zones per 8 well strip, 12 strips per kit).

Test Date:

Test performed by:

Approved By:

Other information:

Kit Lot Number:

Reaction	Dye	Use (see Instructions)	Test Result	Action	Sample Information
1	Red	Positive Control: DNA			
2	Purple	Negative Control: Water			
3	Blue	Zone 1 Inhibition Test			
4	Purple	Zone 1 Wipe Test			
5	Blue	Zone 2 Inhibition Test			
6	Purple	Zone 2 Wipe Test			
7	Blue	Zone 3 Inhibition Test			
8	Purple	Zone 3 Wipe Test			

13. Guide to Symbols Used

	Contains sufficient for N Tests		Lot Number
	Consult Instructions for Use		Catalogue Number
	Site of Manufacture		Temperature Limit
	Use by YYYY-MM-DD		Distributed by
	Warnings and Cautions		
	Global Trade Item Number		

14. Manufacturer Contact Details

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