



Instructions for Use Biofortuna SSPGo™ HLA No Template Control Kit BF-40-02

CE revision 2, July 2014

1. Intended Use

The Biofortuna SSPGo No Template Control (NTC) Kit contains supplementary tests for PCR amplicon contamination to be used in conjunction with Biofortuna SSPGo kits that do not have an integral NTC.

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. The NTC test is used to detect potential PCR or DNA contamination present in the DNA diluent used for a test sample.

3. Test Description

Each SSPGo No Template Control kit consists of 12 strips of 8 PCR reactions containing freeze-dried PCR buffer, polymerase and primers specific for the HLA-DRA gene and will produce an 187bp amplicon from human genomic DNA or amplified DNA. All Biofortuna kits utilise a DRA amplicon as an internal control, therefore any contamination from the use of Biofortuna SSPGo kits will have co-amplification of the DRA gene, and will be detected by the NTC test primers.

4. Kit Contents

- 12 strips of 8 PCR reactions, each containing pre-dispensed freeze dried primers, polymerase, dNTPs* and buffer. The eight reaction strip format is shown below.

Reaction	Dye	Use
1	Red	No Template Control: Sample 1
2	Purple	No Template Control: Sample 2
3	Purple	No Template Control: Sample 3
4	Purple	No Template Control: Sample 4
5	Purple	No Template Control: Sample 5
6	Purple	No Template Control: Sample 6
7	Purple	No Template Control: Sample 7
8	Purple	No Template Control: Sample 8

- 12x8 PCR caps
- 1x instructions for use
- 1x 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.
- 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 interpretation of 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 kits should be stored at 2-28°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 or if there is no desiccant bag present.

Using the sealing sheets or caps provided only, ensure PCR vessels are sealed tightly after adding DNA as omitting this may lead to evaporation during PCR amplification. Pay particular attention to edges and corners.

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 DNA at a temperature of up to 21°C and humidity of no more than 60%.



Note (2): 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 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 use a known contamination free pipette to load sample into PCR reactions, including this NTC test.

1. Open the foil pouch, and label appropriately; one strip can be used to test between one and eight samples. Once opened the PCR reactions should be used within 3 hours.
2. For each DNA sample genotyped add 10µl of the water or diluent used to one of the reactions of the NTC strip.
3. If a positive control is required use 10µl of 10ng/µl human genomic DNA. This will result in a positive test amplicon of 187bp.
4. Cap the reactions with the provided caps and proceed with the standard SSPGo PCR parameters as shown below.

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

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. 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	←	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

Record the results using the sample record table on page 6 of this IFU. A 187bp amplicon will be observed if there is SSPGo 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. A positive result for any diluent sample indicates that the genotyping of that sample is invalid and should be repeated with another DNA sample using different reagents.

10. Quality Assurance and Control

Assay testing: Using PCR amplicon from a Biofortuna Kit, the NTC Test was performed on the amplicon undiluted and then in dilutions from 1×10^1 to 1×10^{15} . The amplicon was detected in dilutions up to and including 1×10^{15} .

The NTC Test was performed on gDNA at 100ng/ μ l and then in dilutions from 1×10^1 to 1×10^{15} . The DNA was detected in dilutions up to 1×10^3 .

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. Biofortuna NTC Test Sample Record Sheet

It is recommended that this sample record sheet is photocopied prior to use as the NTC Kit has sufficient tests for 96 samples (twelve strips of eight tests).

Sample 1 Description. _____

Sample 2 Description. _____

Sample 3 Description. _____

Sample 4 Description. _____

Sample 5 Description. _____

Sample 6 Description. _____

Sample 7 Description. _____







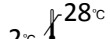



Sample 8 Description. _____

NTC Sample record

Reaction	Dye	Sample ID	Date of test	Result
1	Red			
2	Purple			
3	Purple			
4	Purple			
5	Purple			
6	Purple			
7	Purple			
8	Purple			



13. Guide to Symbols Used

	Number of Tests
	EC Representative
	Consult Instructions for Use
	Site of Manufacture
	In Vitro Diagnostic
	Expiry Date YYYY-MM-DD
	Storage Temperature
	Lot Number
	Distributed by
	Global Trade Item Number

14. Manufacturer Contact Details

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15. Translations

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