



HFE Box 2.0

Typing Kit 1.1

In vitro diagnostics disposal

Instructions Manual



DESENVOLVIMENTO E PRODUÇÃO
DE TESTES DE DIAGNÓSTICO

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RESEARCH AND DEVELOPMENT OF
DIAGNOSTIC TESTS

Version 1.2; Maio de 2010



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Material Safety Data Sheet (MSDS) (2/3)

12. Ecological information

No data available.

13. Waste disposal information

Waste disposes in accordance with all applicable regulations (the disposals should be incinerated).

14. Transport information

During transportation the temperature could not exceed 25°C.
Transportation should not exceed 3 days.

15. Other information

The above information is based on our current level of knowledge, but does not purport to be all-inclusive and shall be used only as a guide. *geneBOX - R&D Diagnostic Tests* shall not be held liable for any damage resulting from handling or from contact with the above products.

**If your problems persist, do not hesitate to contact our
technical support**

+351 231 410 946

Material Safety Data Sheet (MSDS) (2/3)

6. Personal protection.

Hand protection: Wear appropriate chemically resistant gloves.

Eye protection: Chemical safety goggles are recommended.

Skin protection: Wear laboratory coat.

7. Handling and storage

Handling: Avoid substance contact.

Storage: Protect from light. Store at temperature indicated on package.

Package Damage: reject damaged components.

8. Hazards

Master Mix Components: may be harmful by **inhalation, ingestion or skin absorption**. May cause eye and skin irritation. Material is irritating to mucous membranes and upper respiratory tract. **Ingestion** of large amounts can cause stomach pains, vomiting or diarrhea.

9. First aid measures

In case of eye contact: Immediately flush eyes with large amounts of water for at least 15 minutes. Call a physician.

In case of skin contact: Immediately wash skin with soap and large amounts of water. Wash contaminated clothing before re-use.

In case of ingestion: Wash out mouth with water provided person is conscious. Call a physician if needed.

In case of inhalation: remove to fresh air, if not breathing give artificial respiration. If breathing difficult, give oxygen. Call a physician.

10. Fire fighting measures

Extinguishing media: Water, carbon dioxide, dry chemical powder or appropriate foam.

Extinguishing media NOT to use: None are known.

Special exposure hazards: May emit toxic fumes of carbon dioxide, carbon monoxide, nitrogen, phosphorus, hydrogen chloride, and hydrogen gas under fire conditions.

Special fire-fighting equipment: When large amounts of substances are released work only with self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

11. Accidental release measures

Personal Precautions: Avoid substance contact. No further requirements.

Cleaning Method: Clean up affected area. No further requirements.

Presentation

This kit contains typing plates with dried primers mixes and PCR Master Mix for genotyping of polymorphisms on: codon 63, 65, 105 and 282, introns 1, 2, 4 e 5 and 5' UTR of HFE.

Product Changes and Improvements

The HFE Box specificity and interpretation tables are constantly updated, to include new HFE mutations described. This product can also be improved in order to increase the yield of the specific PCR product.

The primers exchanged, added or modified, compared to the previous lot, are detailed in the table below.

Tube	primers	motivation
N/A		

Quality Control

The specificity of each primer solution of the kit has been tested using DNA samples from Genebox. Positive and negative results were obtained for each mutation. The HFE Box 2.0 Typing kit 1.1 has the Genebox quality warranty.

HFE Box 2.0 Typing Kit 1.1 Components

- **HFE typing plate⁺** (48 typings)
12 plates (4 samples each) (Keep at -30/-15 °C)
- **PCR Master Mix (With Taq DNA Polymerase)**
12 X 320 µl (keep at -30/-15°C)
- **PCR plate sealers**
12 sealers
- **Instructions Manual**
1 Instructions Manual

⁺ With dried specific primers pares.

PCR Master Mix Components

Nucleotides

Final concentration of each dNTP: 600 µM

PCR Buffer

Final concentration: 3,3x NH₄ Buffer; 2,0 mM MgCl₂ and 0,4 U/µl Taq DNA polymerase, pH 8.3.

Glycerol

Final concentration: 16,6%

Cresol Red

Final concentration: 300µg/ml

Material Safety Data Sheet (MSDS) (1/3)

geneBOX - R&D Diagnostic Tests™ PCR-SSP Kits

geneBOX™ PCR-SSP typing products

This Material Safety Data Sheet (MSDS) applies to all geneBOX - R&D Diagnostic Tests SSP™ typing kits

1. Chemical products and company identification

Date of Issue:	May 2010
Product group:	geneBOX™ PCR-SSP Typing Products
Manufacturer:	geneBOX - R&D Diagnostic Tests, Biocant- centro de inovação em biotecnologia núcleo 4, lote 3 3060-197 Cantanhede, Portugal
tel/fax:	+ 351 231 410 946/ +351 231 410 947
e-mail:	info@genebox.com

2. Composition and reagents information

Component	Chemical	Common Name
Plate	Deoxyribonucleic acid Cresol Red	Oligonucleotide
PCR Master Mix	Deoxyribonucleotides NH ₄ Buffer Magnesium chloride Cresol Red Glycerol Taq DNA Polymerase	Nucleotides MgCl ₂ Glycerine Taq

3. Physic-chemical properties:

Components	Appearance	Colour	Odour
Plate	dried, in plate wells	Red	none
Master Mix	liquid	Pink/red	none

4. Toxicological information

Chemical	Toxicities
Glycerol	LD50= oral 4090 mg/kg (mouse) LD50= oral 12600 mg/kg (rat) LD50= oral 1480 mg/kg (human)

5. Stability and reactivity

Conditions to avoid: Heat and moisture.

Incompatibilities: Strong oxidizing agents, strong bases.

Declaration of conformity

Product Name: HFE Box

Product Number: GB.01.08

Intended use: Genotyping of HFE gene.

Manufacturer: geneBOX - R&D Diagnostic Tests,
biocant – centro de inovação em biotecnologia
núcleo 4, lote 3
3060-197 cantanhede, portugal

We, geneBOX - R&D Diagnostic Tests, hereby declare that this product, to which this declaration of conformity relates, is in conformity with the following standards and other normative documents ISO 9001:2008 and ISO 13485:2004, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, conformity assess using Annex IV, as transposed into the national laws of the Member States of the European Union.

The technical file of the product is maintained at geneBOX - R&D Diagnostic Tests, biocant, centro de inovação em biotecnologia, 3060-197 Cantanhede, Portugal.



Sandra Balseiro
Directora Técnica

PCR amplification protocol

Reagents

- DNA Sample (100-200 ng/μl)
- PCR Master Mix
- ddH₂O (not supplied)

DNA Extraction

For SSP typing highly pure DNA is needed. We recommend isolation of DNA using any extraction kit with CE marking, which guarantees an OD ratio 260/280 higher than 1.6 and a 100ng – 200 ng/μl DNA concentration.

Alternatively, the DNA can be extracted using trimethylammonium-bromide salts (DTAB/CTAB) or by salting out, dissolving it in TE Buffer. The same OD and concentration values should be assured.
DO NOT USE HEPARINISED BLOOD WITH THIS METHOD

PCR Amplification

1. Spin briefly the DNA and Master Mix tubes.
2. Add:
 - **72 μl of PCR Master Mix,**
 - **150 μl of ddH₂O, and**to a 0,7 ml or 1,5 ml tube.
3. Vortex the tube vigorously for 15s.
4. Load **10 μl** of the mix into the negative control well.
5. Add **19 μl** of **DNA sample** (conc. 100-200 ng / μl) to the mix tube.
6. Vortex the tube vigorously for 15s.
7. Load **10 μl** of the mix into each tube of the plate (**20 primers pairs and 1 positive control**).

8. Repeat the previous steps for other DNA sample to complete the HFE typing plate.
9. Close the typing plate with a self-adhesive lid and put it in a 96 well thermo cycler.

PCR Cycling Parameters

Step	Temperature	time	Cycle
Denaturation	96 °C	1 min	1
Denaturation	96 °C	25 sec	5
Annealing	70 °C	45 sec	
Extension	72 °C	30 sec	
Denaturation	96 °C	25 sec	21
Annealing	65 °C	45 sec	
Extension	72 °C	30 sec	
Denaturation	96 °C	25 sec	4
Annealing	55 °C	1 min	
Extension	72 °C	2 min	
Extension	72 °C	10 min	1
Keep (optional)	4 °C	Infinite	1

10. Keep the plate at 2-8 °C after the PCR have finished.
11. Detect the PCR products with 2% agarose gel electrophoresis.

Warranty

geneBOX - R&D Diagnostic Tests warrants its products to the client against defects in materials and contents under normal application. The company products under this warranty shall be replaced, at no charge, to the damaged client.

This warranty applies only to products that have been handled and stored in accordance with its recommendations/specifications.

The claims must be posted directly to geneBOX in writing and must be accompanied by a copy of the purchaser's invoice.

This product may not be reformulated, repacked or resold in any form without geneBOX - R&D Diagnostic Tests consent.

Guarantee

geneBOX - R&D Diagnostic Tests guarantees that the primers in HFE Box typing kit have the specificities given in the Results Interpretations Sheet/Tables of the product insert.

1. Typing plate

When stored at -20°C, the dried primers are stable for 12 to 19 months from the date of manufacture (see lot validity in the package).

When stored at 4°C, the dried primers are stable for 12 months from the date of manufacture (see lot validity in the package).

At room temperature, the dried primers are stable for 3 to 4 weeks from the date of the reception.

When the sealer is removed the dried primers shall be stable for 2 days, maximum, in dried conditions.

2. PCR Master Mix

When stored at -20°C, the PCR Master Mix is stable for 18 months from the date of manufacture (see lot validity in the package).

When stored at 4°C, the Master mix is stable for 15 days from the date of the reception.

At room temperature, the master mix shall be stable for 3 days from the date of the reception.

The master mix should not be left or stored with the cap open.

3. DNA

Using extracted DNA from salting out or any kit procedure the samples should be stored at 4°C or -20°C. If you chose to freeze the samples you must avoid repeated cycles of heating/freezing, in order to preserve your sample stability.

The DNA samples stored in dH₂O are stable for at least 2 to 4 weeks (at 4°C) or 24 months (at -20°C).

The DNA samples stored in buffer are stable for at least 12 months (at 4°C) or 5 years (at -20°C).

Gel Electrophoresis protocol

Preparing 2% agarose gel

1. Dissolve **4 grams** of electrophoresis **grade agarose** powder in **200 ml** of **1X TAE buffer**.
2. Melt the agarose powder completely in a microwave oven.
3. Cool the heated agarose gel to **~ 50°C**.
4. Add at least 10 µl of **ethidium bromide⁺⁺** (10 mg/ml) or **Sybr SafeTM** (100000 x concentrate) to the heated agarose. Stir until it is thoroughly incorporated.
5. On a balanced surface, set up a gel plate with **96 wells**.
6. Cast a **5mm** thick gel on the plate.
7. Allow the gel to settle.

⁺⁺**Caution**, this reagent is a strong mutagenic agent (read carefully its MSDS before using it).

Gel Electrophoresis

1. Submerge the gel in 1X TAE buffer in a gel box.
2. Gently remove the caps to avoid splashing of PCR products.
3. Load 10 µl into each well on the gel.
4. Connect the electric leads and turn on the power supply (115V). Electrophoresis for ~ 20 minutes, or until 2/3 of the lane.
5. Transfer the gel onto a UV transilluminator, document the result by photography.
6. Use the **result interpretation table** to interpret results.

HFE Box 2.0 plate

H	G	F	E	D	C	B	A	
								1
								2
		NC	PC					3
								4
								5
		NC	PC					6
								7
								8
		NC	PC					9
								10
								11
		NC	PC					12

Technical Guide

1. DNA Quality and Concentration

For optimal results with the HFE Box 2.0 Typing Kit 1.1™ the quality of DNA is critical. Good quality DNA means an OD ratio 260/280 higher than 1.6 and the major portion of DNA should run higher than 9.4 kb on an agarose gel. Different quality and concentration values require DNA re-extraction.

The quantity of DNA should be 100ng – 200 ng/μl. Excess of DNA can cause unspecific amplification.

We recommend any DNA extraction kit which has CE marking, in order to obtain this highly DNA purity

2. Taq Polymerase

HFE Box 2.0 Typing Kit 1.1™ kits have been intensively tested with the Taq DNA Reagente 5 (Reagente 5, Lisboa, Portugal).

3. PCR Master Mix

For optimal results with the HFE Box 2.0 Typing Kit 1.1™ the use of master mix supplied is obligatory.

4. Amplification Procedure

At the end of PCR, examine the degree of evaporation and condensation of PCR reaction mixture. If there is more than 20% volume loss do not validate the results. In order to prevent this you should overlay the PCR reaction mixture with mineral oil or use a MicroMat. It is also a good practice to maintain QC records on the heating lid.

If the temperature of the heating lid is not high enough, it will cause condensation problems on the lid.

5. Thermal Cycler

We recommend the use of any thermocycler with the following characteristics:

- heating rate up to 2.5°C/sec; cooling rate up to 1.5°C/sec; temperature range 4-100°C; temperature uniformity ±0.5°C; heated lid up to 100°C.

6. Validity

As specified in the package

**If your problems persist, do not hesitate to contact our
technical support
+351 231 410 946**

Precautions and Warnings

PCR amplification allows the amplification of small quantities of sample DNA in an exponential way. However, this is also true for foreign DNA, which can contaminate our PCR method. Consequently, special laboratory practices are necessary in order to avoid false positive amplifications. Bellow is listed Genebox recommendations to circumvent contaminations:

- Work in separated pre-PCR and post-PCR rooms.
- Laboratory workflow must be unidirectional, from pre-PCR to post-PCR area.
- Specific equipment for each working area must be used (sample preparation, preamplification, amplification and postamplification).
- All equipment used in post-PCR should not leave this area.
- Use dedicated micropipettes, gloves and lab coats in each area.
- Use non talcum powder gloves (since talcum could inhibit the PCR reaction).
- Use filter tips in order to avoid cross contamination.
- Check regularly micropipettes, in order to ensure that they are accurate within 5 % of fixed volume.
- Use different micropipettes depending on the volume we wish to load.
- Check regularly thermocyclers, in order to ensure that they are accurate within 1% of fixed temperature.
- Open and close reagent vials carefully. After use, close vials and store at indicated temperatures.
- Do not use a kit after its expiration date.
- packaging material included within the kit is resistant to the indicated storage conditions. Storage at different conditions can cause breakage of the material, and possible contamination of the kit reagents.
- plastic material included within the kit is resistant under normal conditions of use. Use of plastic material in extreme conditions may cause its breakage, and therefore, the impossibility to use the kit.
- check suitability of DNA quantity and quality before use the kit.

General instructions for laboratory safety:

- do not eat, drink or smoke in laboratory work areas
- wear disposable gloves
- wear clean lab coats and eye protection
- wash hands thoroughly after handling specimens and test reagents
- clean the working area before and after kit handling.
- do not pipette by mouth.

HFE Box 2.0 plate identification

Position (sample 1)	Position (sample 2)	Position (sample 3)	Position (sample 4)	Gene	Polymorphic site
1A	4A	7A	10A	HFE	5' UTR (-7T>C)
1B	4B	7B	10B	HFE	5' UTR (-7T>C)
1C	4C	7C	10C	HFE	Intron 1 (rs2858993)
1D	4D	7D	10D	HFE	Intron1 (rs2858993)
1E	4E	7E	10E	HFE	Intron1 (rs2227837)
1F	4F	7F	10F	HFE	Intron 1 (rs2227837)
1G	4G	7G	10G	HFE	Intron 2 (rs2071303)
1H	4H	7H	10H	HFE	Intron 2 (rs2071303)
2A	5A	8A	11A	HFE	Intron 4 (rs1800708)
2B	5B	8B	11B	HFE	Intron 4 (rs1800708)
2C	5C	8C	11C	HFE	Intron 5 (rs1572982)
2D	5D	8D	11D	HFE	Intron 5 (rs1572982)
2E	5E	8E	11E	HFE	H63D (C187G)
2F	5F	8F	11F	HFE	H63D (C187G)
2G	5G	8G	11G	HFE	S65C (A193T)
2H	5H	8H	11H	HFE	S65C (A193T)
3A	6A	9A	12A	HFE	I105T (T314C)
3B	6B	9B	12B	HFE	I105T (T314C)
3C	6C	9C	12C	HFE	C282Y (G845A)
3D	6D	9D	12D	HFE	C282Y (G845A)
3E	6E	9E	12E	PC	-----
3F	6F	9F	12F	NC	-----

Results interpretation table

Mix	Mix	Mix	Mix	Gene	Polymorphism	Allele	Specific band	Control band**
1A	4A	7A	10A	HFE	5' UTR (-7T>C)	T	269	790
1B	4B	7B	10B	HFE	5' UTR (-7T>C)	C	269	790
1C	4C	7C	10C	HFE	Intron 1 (rs2858993)	A	218	790
1D	4D	7D	10D	HFE	Intron 1 (rs2858993)	T	218	790
1E	4E	7E	10E	HFE	Intron 1 (rs2227837)	T	181	790
1F	4F	7F	10F	HFE	Intron 1 (rs2227837)	C	181	790
1G	4G	7G	10G	HFE	Intron 2 (rs2071303)	A	259	790
1H	4H	7H	10H	HFE	Intron 2 (rs2071303)	G	259	790
2A	5A	8A	11A	HFE	Intron 4 (rs1800708)	T	188	790
2B	5B	8B	11B	HFE	Intron 4 (rs1800708)	C	188	790
2C	5C	8C	11C	HFE	Intron 5 (rs1572982)	G	329	790
2D	5D	8D	11D	HFE	Intron 5 (rs1572982)	A	329	790
2E	5E	8E	11E	HFE	H63D (C187G)	His (C)	274	790
2F	5F	8F	11F	HFE	H63D (C187G)	Asp (G)	274	790
2G	5G	8G	11G	HFE	S65C (A193T)	Ser (A)	188	790
2H	5H	8H	11H	HFE	S65C (A193T)	Cys (T)	188	790
3A	6A	9A	12A	HFE	I105T (T314C)	Ile (T)	316	790
3B	6B	9B	12B	HFE	I105T (T314C)	Thr (C)	316	790
3C	6C	9C	12C	HFE	C282Y (G845A)	Cys (G)	258	790
3D	6D	9D	12D	HFE	C282Y (G845A)	Tyr (A)	258	790
3E	6E	9E	12E	HFE	Positive control	-	-	790
3F	6F	9F	12F	HFE	Negative control	-	-	-
DNA 1	DNA 2	DNA 3	DNA 4					

**Control primer pairs match with non-allelic sequences. The internal positive control primer pairs amplify segments of the gene PIC1, giving rise to 790 base pair fragments. In the presence of the specific band amplification the control band intensity often decreases. The PCR reaction is only valid in the presence of control band or, in some cases, in the presence of the specific band. In the absence of the control band, please repeat the typing. If the PCR reaction results in fragments different from the specific or control band please do not consider, they are unspecific fragments.

Troubleshooting Guide

PROBLEMS	POSSIBLE CAUSES	SUGGESTIONS
The control and specific bands are weak.	Concentration of DNA sample is too low.	Check DNA quality and concentration
		Re-extract the DNA sample or try not add water into the PCR Mix
	DNA polymerase inhibitors in the DNA sample	Repeat typing with a good quality DNA sample
		Re-purify the sample DNA
Missing internal control bands in one or several lanes.	DNA polymerase inhibitors in the DNA sample.	Repeat typing with a good quality DNA sample
		Re-purify the sample DNA
	Dried PCR amplification products	Check the plate sealing
		Repeat the typing using a PCR MicroMat and/or overlay the PCR reaction mix with mineral oil
False negative of a specific band while the internal control appears normal	Degradation of DNA sample	Re-extract the DNA sample with fresh material
		Repeat typing with a good quality DNA sample
More than two specific alleles are detected/ Ambiguous results	Excess of template DNA	Check DNA quality and concentration
		Dissolve the DNA sample in ddH_2O in order to have the proper concentration
		Repeat typing with a good quality DNA sample
	Contamination with previously amplified PCR products or with other DNA samples during the DNA extraction or PCR preparation steps	Clean the working area
		Work in separated pre-PCR and post-PCR rooms
		Keep different lab coats in pre-PCR and post-PCR rooms
		Change protective gloves frequently
		Repeat typing with a good quality DNA sample
Blurred bands	Degradation of DNA sample	Re-extract the DNA sample with fresh material
		Repeat typing with a good quality DNA sample
	Excess of template DNA	Check DNA quality and concentration
		Dissolve the DNA sample in ddH_2O in order to have the proper concentration
		Repeat typing with a good quality DNA sample
	Electrophoresis Buffer Problems: wrong buffer or older buffer	Use a fresh recommended buffer