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CE 0197

geneBOX
DESENVOLVIMENTO E PRODUÇÃO DE TESTES DE DIAGNÓSTICO

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Product Code: 1207

HLA-B*57 Single Box 1.0 Typing Kit

In vitro diagnostics disposal

Instructions Manual

geneBOX
DESENVOLVIMENTO E PRODUÇÃO DE TESTES DE DIAGNÓSTICO

Version 1.4; May 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 25ºC.
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

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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 diarrhoea.

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

Some HLA-B*57 subtypes are associated with the rate of illness progressions in HIV-1 infected patients, particularly B*5701 allele in Caucasians and B*5703 allele in Africans populations. The presence of these alleles is strongly associated with hypersensitivity related with ABACAVIR administration/treatment.
HLA-B*57 is also related with HCV infection and *psoriases* susceptibility.

This kit contains typing plates with dried primer mix and PCR Master Mix for rapid screening of HLA-B*57 gene.

Product Changes and Improvements

The HLA-B*57 Single Box specificity and interpretation tables are constantly updated, to include new HLA class I alleles 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 following 3 DNA samples from the *13th International Histocompatibility Workshop SSOP Panel* were used to verify the kit primers mixes specificity.

Workshop Name	Designation
IHW 09052	DBB
IHW 09273	LADA
IHW 09398	FH18

No false positive or negative amplifications were obtained.

The negative control tube can detect cross-contamination with PCR products.

Cell line validation sheet

HLA-B*57 low resolution SSP typing kit					
Cell line		Cell Typing			HLA-B*57
		HLA-A*	HLA-B*	HLA-Cw*	Positive well no.
9273	LADA	0201;8001	0702;5703	0701;0802	All except negative control
9052	DBB	0201	5701	0602	All except negative control
9398	FH18	7401;3601	5301;5703	0401;0701	All except negative control

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	Oligonucleotide
	Cresol Red	
PCR Master Mix	Deoxyribonucleotides	Nucleotides
	NH_4 Buffer	
	Magnesium chloride	MgCl2
	Cresol Red	
	Glycerol	Glycerine

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: HLA-B*57 Single Box

Product Number: GB.12.07

Intended use: HLA-B*57 low resolution histocompatibility testing.

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 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices 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 Park, Parque tecnológico de Cantanhede, 3060-197 Cantanhede, Portugal.

Sandra Balseiro
Technical Director

HLA-B*57 Single Box 1.0 Typing Kit Components

- **HLA-B*57 Single Box typing plates**[+] (88 typing)

  4 plates (22 samples each) (Store at -30 to -15 ºC)

- **PCR Master Mix (With Taq DNA Polymerase)**

  4 X 80 µl (Store at -30 to -15ºC)

- **Plate sealers**

  6 sealer caps

- **Instructions Manual**

  1 Instructions manual

[+] With dried specific primers pairs (1 specific primer pair; 1 negative control and 1 positive control).

PCR Master Mix Components

Nucleotides
Final concentration of each dNTP: 600 µM
PCR Buffer
Final concentration: 3,3x NH_4 Buffer; 2,0 mM $MgCl_2$ and 0,4 U/µl Taq DNA polymerase, pH 8.3.
Glycerol
Final concentration: 16,6%
Cresol Red
Final concentration: 300µg/ml

PCR amplification protocol

Reagents

- DNA Sample (100-200 ng/µl)
- PCR Master Mix
- $_{dd}H_2O$ (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 DNA concentration between 100ng – 200ng/µl.
Alternatively, the DNA can be extracted using trimethyammoium-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:
   - **80 µl of PCR Master Mix,**
   - **160 µl of ddH_2O**

   to a 0,7 ml or 1,5 ml tube.
3. Vortex the tube vigorously for 15s.
4. Load **9 µl** of the mix into each tube of the strip (**1 primers pair**).
5. Load **1 µl of DNA sample (conc. 100-200 ng/µl)** in each well (except to the control wells).
6. Close the typing plate with a strip cap (sealer cap) and put it in a 96 well thermo cycler.

PCR Cycling Parameters

Step	Temperature	time	Cycle
Denaturation	96 ºC	1 min	1

Technical Guide

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, amplification and preamplification).
- 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.

Denaturation	96 ºC	25 sec
Annealing	70 ºC	45 sec
Extension	72 ºC	30 sec
		5

Denaturation	96 ºC	25 sec
Annealing	65 ºC	45 sec
Extension	72 ºC	30 sec
		21

Denaturation	96 ºC	25 sec
Annealing	55 ºC	1 min
Extension	72 ºC	2 min
		4

Extension	72 ºC	10 min
		1

Keep (optional)	4 ºC	Infinite
		1

7. Keep the plate at 2-8 ºC after the PCR have finished.
8. Detect the PCR products with 2% agarose gel electrophoresis.

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 $_{dd}$H2O 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

Troubleshooting Guide

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

Repeat typing with a good quality DNA sample

DNA polymerase inhibitors in the DNA sample
Re-purify the sample DNA

Repeat typing with a good quality DNA sample

Missing internal control bands in one or several lanes.
DNA polymerase inhibitors in the DNA sample.
Re-purify the sample DNA

Repeat typing with a good quality DNA sample

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 $_{dd}$H2O 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

Gel Electrophoresis protocol

PREPARING 2% AGAROSE GEL

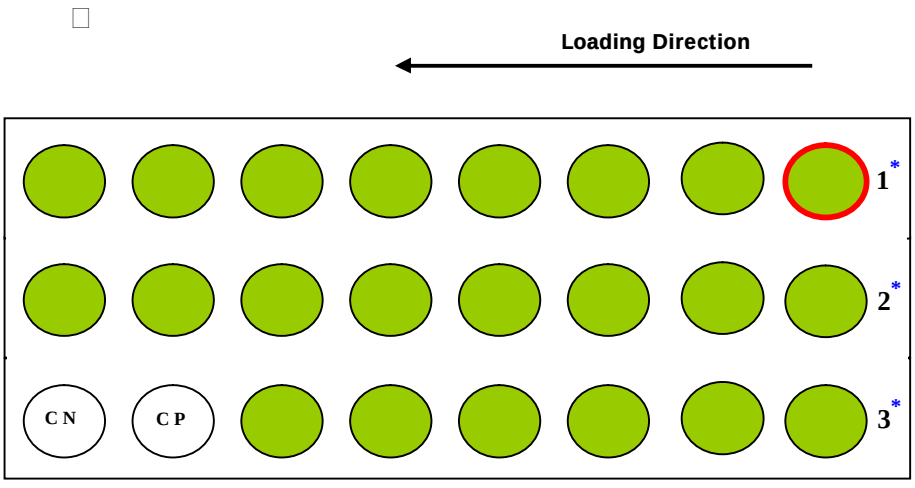
1. Dissolve **4 grams** of electrophoresis **grade agarose** powder in **200 ml** of **1X TAE buffer**.
2. Melt the agarose powder completely in a microwave oven.
3. Cool the heated agarose gel to **~ 50°C.**
4. Add at least 10 µl of **ethidium bromide**[++] (10 mg/ml) or **Sybr Safe**[TM] (100000 x 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 sheet*** to interpret results.

HLA-B*57 Single Box 1.0 plate



* Numbers may differ from plate to plate:
The no 1 could be 4, 7, 10
The no 2 could be 5, 8, 11
The no 3 could be 6, 9, 12

HLA-B*57 Single Box 1.0 Plate Identification

Position
Product

Lines 1, 2 e 3 (except controls)
B*5701-5714 except 5705

CP
Positive control

CN
Negative control

Results Interpretation sheet

Position
HLA-B 57
Specific band
Control band**

Lines 1,2
and
3 (except controls)
B*57 positive
380
790 pb

B*57 negative
----
790 pb

PC
Positive control
380

NC
Negative control
----
790 pb

****Control primer pares match with non-allelic sequences. The internal positive control primer pairs amplify segments of the HLA-DRB1 gene, giving rise to 790 base pair fragment.**
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.

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