Product Code: 1306

HLA-B Single Box 1.0 Typing Kit

In vitro diagnostics disposal

Instructions Manual

Version 1.6, May 2010

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Presentation

This kit contains typing plates with dried primers mixes and PCR Master Mix for low resolution typing of HLA-B gene.

Product Changes and Improvements

The HLA-B 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 specificity of each primer solution of the kit has been tested using 51 DNA samples from the *IHWG Sequence Polymorphism Reference DNA SSOP Panel* (see cell line validation sheet).
No false positive or negative amplifications were obtained.
The negative control tube can detect cross-contamination with PCR products.

Cell line validation sheet

Typing kit SSP HLA-B low resolution			
Cell line		Cell typing HLA-B*	HLA-B Well number
9215	M7	3501;5301	27/28/29/41/42
9273	LADA	0702;5703	5/13/18/41/42
9263	G085	4006;5201	1/2/4/31/41/42
9373	FH1	1402;5801	11/19/41/42
9030	JHAF	51011	1/2/3/41
9035	JBush	3801	15/41
9045	TUBO	51011	1/2/3/41
9220	XLI-ND	1302;4006	10/31/41/42
9077	T7527	4601	13/36/42
9085	EJ32B	1801	21/42
9103	KT14	4006;51011	1/2/3/31/41/42
9374	FH2	4001;4402	7/8/31/32/33/41/42
9375	FH3	1402;3502	11/27/28/29/42
9364	GRC202	3505;4004	27/29/31/42
9371	ISH4	1501;4601	12/13/36/42
9368	280599	3901;3802	15/16/41/42
9367	LCK	38021;4601	13/15/36/41/42
9394	BPOT	0703;15	5/12/13/42
9048	LBUF	1302	10/41
9032	BSM	1501	12/13/42
9237	APA	1502;5502	13/24/42
9253	THAI742	1512;4601	12/13/36/42
9369	ISH3	1526N	12/13/42
9380	FH6	2702;0705/6	5/26/41/42
9376	FH4	2703;2705	26/41
9266	PAR	2706;4801	26/33/41/42
9377	FH5	2709;4403	7/8/26/41
9068	BM9	3501	27/28/29/42
9056	K0SE	3503	27/28/29/42
9009	KAS011	3701	30/41
9381	FH7	3908;1801	16/21/42
9385	FH11	4404;07021	5/7/8/41
9047	PLH	4701	37/41
9392	GN00218	4703	37/41
9040	BM15	4901	23/41
9092	BM92	51011	1/2/3/41
9370	230699	5103;07021	1/2/3/5/41/42
9372	ISH5	5401;4801	24/33/38/42
9052	DBB	5701	18/41
9267	LE023	7301;51011	1/2/3/39/42
9382	FH8	8201;27052	24/26/41/42
9366	Daudi	5801;5802	19/41
9014	MGAR	0801	6/42
9053	HOR	44031	7/8/41
9021	RSH	4201	35/42
9024	KT17	15011;3501	12/13/27/28/29/42
9016	RML	51011	1/2/3/41
9297	HAG	4102	33/42
9386	FH12	4402;27052	7/8/26/41
9387	FH13	44031;1501	7/8/12/13/41/42
9398	FH18	5301;5703	20/18/28/41

HLA-B Single Box 1.0 Typing Kit Components

- **HLA-B single box typing plate[+]** **(24 typings)**

  12 plates (2 samples each) (Keep at -30/-15 ºC)

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

  12 X 310 µl (keep at -30/-15ºC)

- **Plate sealers**

  12 PCR plate sealers

- **Instructions Manual**

  1 Instructions manual

[+] With dried specific primers pares (42 specific primers).

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 100ng – 200 ng/µl DNA concentration.
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:
   - **153 µl of PCR Master Mix,**
   - **308 µl of ddH_2O,**

   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 **40 µl of DNA sample (conc. 100-200 ng / µl)** to the PCR mix tube.
6. Load **10 µl** of the mix into each tube of the plate (**43 primers pairs and positive control**).
7. Repeat the previous steeps for other DNA sample to complete the HLA-B typing plate.

8. 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 ºC	1 min	1
Denaturation Annealing Extension	96 ºC 70 ºC 72 ºC	25 sec 45 sec 30 sec	5
Denaturation Annealing Extension	96 ºC 65 ºC 72 ºC	25 sec 45 sec 30 sec	21
Denaturation Annealing Extension	96 ºC 55 ºC 72 ºC	25 sec 1 min 2 min	4
Extension	72 ºC	10 min	1
Keep (optional)	4 ºC	Infinite	1

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

Gel Electrophoresis protocol

PREPARING 2% AGAROSE GEL

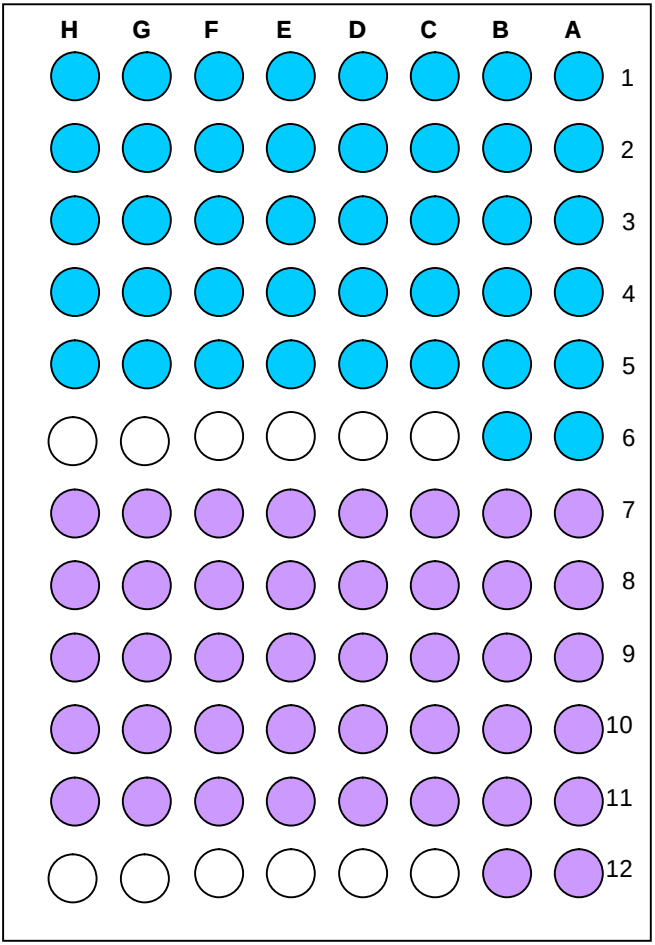
1. Dissolve **4 grams** of electrophoresis **grade agarose** powder in **200 ml** of **1X TAE buffer**.
2. Melt the agarose powder completely in a microwave oven.
3. Cool the heated agarose gel to **~ 50°C.**
4. Add at least 10 µl of **ethidium bromide**[++] (10 mg/ml) or **Sybr Safe**[TM] (100000 x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

HLA-B Single Box 1.0 plate



HLA-B Single Box 1.0 Plate Identification

Well		HLA
1a	7a	B
1b	7b	B
1c	7c	B
1d	7d	B
1e	7e	B
1f	7f	B
1g	7g	B
1h	7h	B
2a	8a	B
2b	8b	B
2c	8c	B
2d	8d	B
2e	8e	B
2f	8f	B
2g	8g	B
2h	8h	B
3a	9a	B
3b	9b	B
3c	9c	B
3d	9d	B
3e	9e	B
3f	9f	B
3g	9g	B
3h	9h	B
4a	10a	B
4b	10b	B
4c	10c	B
4d	10d	B
4e	10e	B
4f	10f	B
4g	10g	B
4h	10h	B
5a	11a	B
5b	11b	B
5c	11c	B
5d	11d	B
5e	11e	B
5f	11f	B
5g	11g	B
5h	11h	B
6a	12a	B
6b	12b	B
6c	12c	Positive control
6d	12d	Negative control
6e	12e	Empty wells
6f	12f	
6g	12g	
6h	12h	

Results Interpretation sheet (1/2)

Well		HLA	Allele	Serotype	ampl	contr**
1a	7a	B	B*5101-23 excepto *5115,*5201/03,*7801-04	**B*51; 52; 78**	504	256
1b	7b	B	B*5101-23 excepto *5110/131/15,*5201-03	**B*51; 52**	401	256
1c	7c	B	B*5101-23 excepto *5110/131/15,*7801/2,*1509	**B*51; 78; 15**	451	256
1d	7d	B	B*5201-03,*15012,*4026,*4028,*5107	**B*52; 15; 40; 51**	440	256
1e	7e	B	B*0702-24 excepto *0713/19/21,*4025, *8101,*3907,*4806, *6812	**B*07; 40; 81; 39; 48; 68**	619	256
1f	7f	B	B*0801-03/06-12,*3510	**B*08; 35**	606	256
1g	7g	B	B*4402-22 excepto *4406/06/15/16	**B*44**	537	256
1h	7h	B	B*4402-22 excepto 4406/16,*4501-03,*1514, *5002	**B*44; 45; 15; 50**	575	256
2a	8a	B	B*4501/03,*4901-03,*5001/02/04,*5402	**B*45; 49; 50; 54**	600	256
2b	8b	B	B*1301/02/06	**B*13**	471	256
2c	8c	B	B*1401-04	**B*14**	390	256
2d	8d	B	B*1501-07/12-15/19/20/24-28/31-36/38-40/43/46/49/50/53/54/55/57/60-62/64,*4003,*4802, *1304,*1801-12,*3520/28, *4003/20,*4417	**B*15; 48; 13; 18; 35; 40; 44**	422	256
2e	8e	B	B*1501/02/04-08/11-17/19-21/24-28/30/32-36/38/39/43/45/48/55-58/60/63,*4601/02, *5701-4,*1303/04,*4405/08	**B*15; 46; 57; 13; 44**	623	256
2f	8f	B	B*1509/10/18/21/23/27/51,*3526,*5122,*7803	**B*15; 35; 51; 78**	562	256
2g	8g	B	B*3801-06,*5119,*3920	**B*38; 51; 39**	500	256
2h	8h	B	B*1548,*3535,*39011-19/22-24,*6701	**B*15; 35; 39; 67**	507	256
3a	9a	B	B*67011/67012,*3910-17/20	**B*67; 39**	548	256
3b	9b	B	B*5701-07 excepto *5705	**B*57**	380	256
3c	9c	B	B*5801/04/05,*5705	**B*58; 57**	345	256
3d	9d	B	B*5801/04/05,*5104,*5301/02/04/06,*1513,*5705, *0712/14/18,*8301(*4406weak?)	**B*58; 51; 53; 15; 57; 07; 83**	319	256
3e	9e	B	B*1801-12	**B*18**	503	256
3f	9f	B	B*4901/03,*5901,*5115,*4418	**B*49; 59; 51; 44**	385	256
3g	9g	B	B*4901/02,*5001/02/04,*4005/15/16/23/26/28/32, *2704,*2706,*2710/15/18/20-22	**B*49; 50; 40; 27**	635	256
3h	9h	B	B*5401/02,*5501-03/05/07/09/10,*5601/02/04/07,*5901, *8201/02,*3917 Cw*15,	**B*54; 55; 56; 59; 82; 39 Cw*15**	490	256
4a	10a	B	B*5601-07,*8201/02,*8301,*0720/24	**B*56; 82; 83; 07**	551	256
4b	10b	B	B*2701-22 excepto *2712/16/18	**B*27**	150	256
4c	10c	B	B*3501-37 excepto *3512/20/22/26/28/31,*5301-06	**B*35; 53**	390	256
DNA1	DNA2					

Results Interpretation sheet (2/2)

well		HLA	Allele	Serotype	ampl	contr**
4d	**10d**	B	B*3501-04/06-09/11/12/14/15/17-21/23-27/29/30/33-36,*5301-06,*1502/13/21/24,*5104,*4406/12,*0712/14/18,*8301	**B*35; 07; 15; 44; 51; 53; 83**	369	256
4e	**10e**	B	B*3501-37 excepto *3501/19/25-27,*18,*7801-04,*1522/59,*5305,*5606	**B*35; 18; 78; 15; 53; 56**	128	256
4f	**10f**	B	B*3701,*4406,*5108	**B*37; 44; 51**	606	256
4g	**10g**	B	B*4001-34 excepto *4012/14/17/21/28,*4701-03	**B*40; 47**	544	256
4h	**10h**	B	B*4001/07/14-16/22N/23/25/30-34,*27053,*3907	**B* 40; 27; 39**	784	256
5a	**11a**	B	B*4001/10/12/14-16/21/22N/23/25/30-34, *4801/03/05/07,*0702/04-07/09-12/14/15/17/18/20/22-24,*8101	**B*40; 48; 07; 81**	608	256
5b	**11b**	B	B*4101-05,*4202,*4405/18,*4501-03,*5002,*27053,*3907	**B*41; 42; 44; 45; 50; 27; 39**	781	256
5c	**11c**	B	B*4201/02,*0801-12,*3510,*3907	**B*42; 08; 35; 39**	702	256
5d	**11d**	B	B*4601/02	**B*46**	459	256
5e	**11e**	B	B*4701-03,*2718,*3702	**B*47; 37; 27**	414	256
5f	**11f**	B	B*5401/02,	**B*54**	508	256
5g	**11g**	B	B*7301	**B*73**	289	256
5h	**11h**	B	B*7801-04, *1509/012,*4026/28,*5605/06	**B*78; 15; 40; 56**	400	256
6a	**12a**	B	Bw4	**B*08; 13; 15; 18; 27; 37; 38; 44; 47; 49; 51; 52; 53; 57; 58; 59**	180	256
6b	**12b**	B	Bw6 excepto B*5401	**B*07; 08; 13; 14; 15; 18; 35; 39; 40; 41; 42; 45; 46; 47; 48; 50; 54; 55; 56; 67; 78; 81; 82; 83**	180	256
6c	**12c**	Positive Control				256
6d	**12d**	Negative Control				
6e	**12e**	Empty wells				
6f	**12f**					
6g	**12g**					
6h	**12h**					
DNA1	**DNA2**					

****Control primer pares match with non-allelic sequences. The internal positive control primer pairs amplify segments of the HLA-DRB1 gene and adenamoutous polyposis coli (PIC1) gene. Giving rise to 1600 + 796 base pair fragments and 256 base pair fragment respectively.**
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.

Results Interpretation Table (1/2)

Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Specific Band	504	401	451	440	619	606	537	575	600	471	390	422	623	562	500	507	548	380	345	319	503	385	635	490	203	150	390	369
B*07					+																							
B*08						+																						
B*13										+																		
B*14											+																	
B*15			*					+				*	*	*						+								+
B*18																					+							
B*27																							+			+		
B*35																											*	*
B*37																												
B*38															+													
B*39																+												
B*40					+							+											+					
B*41																												
B*42																												
B*44							+	+												?								+
B*45								+	+																			
B*46													+															
B*47																												
B*48												+																
B*49									+													+	+					
B*50									+														+					
B*51	+	+	+																	+								+
B*52	+	+		+																+								
B*53																											+	+
B*54																								+				
B*55																								+				
B*56																								+	+			
B*57													+					+										
B*58																			+	+								
B*59																						+		+				
B*67																+	+											
B*73																												
B*78	+		+																									
B*81					+																							

Results Interpretation Table (2/2)

Well No.	29	30	31	32	33	34	35	36	37	38	39	40
Specific Band	128	606	544	784	608	781	702	459	414	508	289	400
B*07												
B*08							?					
B*13												
B*14												
B*15	*											*
B*18	+											
B*27												
B*35	+											
B*37		+										
B*38												
B*39												
B*40			+	*	*							
B*41						+						
B*42							+					
B*44		+										
B*45												
B*46								+				
B*47			+						+			
B*48					+							
B*49												
B*50												
B*51		+										
B*52												
B*53												
B*54										+		
B*55												
B*56												
B*57												
B*58												
B*59												
B*67												
B*73											+	
B*78	+											+
B*81												

* Positive for some subtypes

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
		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
		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

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.

Technical Guide

1. DNA Quality and Concentration

For optimal results with the HLA-B Single Box 1.0 Typing Kit™ 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

HLA-B Single Box 1.0 Typing Kit™ kits have been intensively tested with the Taq DNA Reagente 5 (Reagente 5, Lisboa, Portugal).

3. PCR Master Mix

For optimal results with the HLA-B Single Box 1.0 Typing Kit™ 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 2.5ºC/sec; cooling rate up to 1.5ºC/sec; temperature range 4-100ºC; temperature uniformity ±0.5ºC; heated lid up to 100ºC.

6. Validity

As specified in the package

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

+351 231 410 946

Guarantee

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

1. Typing plate

When stored at -20ºC, the dried primers are stable for 12 to 19 months from the date of manufacture (see lot validity in the package).
When stored at 4ºC, the dried primers are stable for 12 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 steal be stable for 2 days, maximum, in dried conditions.

2. PCR Master Mix

When stored at -20ºC, the PCR Master Mix is stable for 18 months from the date of manufacture (see lot validity in the package).
When stored at 4ºC, the Master mix is stable for 15 days from the date of the reception.
At room temperature, the master mix steal 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 4ºC or -20ºC. 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 dH_2O are stable for 2 to 4 weeks (at 4ºC) or 24 months (at -20ºC).
The DNA samples stored in buffer are stable for 12 months (at 4ºC) or 5 years (at -20ºC).

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.

Declaration of conformity

Product Name: HLA-B Single Box

Product Number: GB.13.06

Intended use: HLA-B 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

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	Nucleotides MgCl2 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.

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.

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

+351 231 410 946

References

1. Bunce M, O'Neill CM, Barnardo MCNM, Krausa P, Browning MJ, Morris PJ, Welsh KI. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilising sequence-specific primers (PCR-SSP*). Tissue Antigens* 1995; 46: 355-367.

2. Bodmer JG, Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Charron, Dupont B, Erlich HA, Fauchet R, Bach B, Mayr WR, Parham P, Sasazuki T, Schreuder GM, Strominger JL, Svejgaard A, Terasaki PI. Nomenclature for factors of the HLA System, 1996. *Hum Immunol* 1997, 53: 98-128.

3. Nomenclature for factors of the HLA System. Compiled by Steven G. E. Marsh for the WHO Nomenclature Committee for Factors of the HLA System. http://www.anthonynolan.com/HIG/nomenc.html

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geneBOX
DESENVOLVIMENTO E PRODUÇÃO DE TESTES DE DIAGNÓSTICO

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