Product Code: 0306

HLA-DR Single Box 1.0

Typing Kit

In vitro diagnostics disposal

Instructions Manual

DESENVOLVIMENTO E PRODUÇÃO
DE TESTES DE DIAGNÓSTICO

Version 1.6; May 2010

CE 0197

geneBOX
DESENVOLVIMENTO E PRODUÇÃO
DE TESTES DE DIAGNÓSTICO

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Presentation

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

Product Changes and Improvements

The HLA-DR Single Box specificity and interpretation tables are constantly updated, to include new HLA-DR 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

HLA-DR low resolution SSP typing kit					
Cell line		Cell Typing			HLA-DRB1
		HLA-A*	HLA-B*	HLA-DRB1*	Positive well no.
9215	M7	0202;0301	3501;5301	03011;0701	6/7/8/12
9273	LADA	0201;8001	0702;5703	09012;1201	14/19/20
9263	G085	0101;2901	4006;5201	1404;15021	9/24/25/27/28
9373	FH1	0205;6802	1402;5801	1302;0102	1/2/5/9/10/18/21
9030	JHAF	31012	51011	0407	11
9035	JBush	3201	3801	1101	16/17
9045	TUBO	0206;0301	51011	1201;1104	16/17/19/20
9220	XLI-ND	0210;3001	1302;4006	0701;09012	12/14
9077	T7527	0207	4601	0901;12	14/19/20
9085	EJ32B	3002	1801	03011	6/7/8
9103	KT14	2402;2602	4006;51011	09012	14
9374	FH2	3401;0201	4001;4402	1302;1301	9/10/18/21
9375	FH3	3301;3101	1402;3502	1101;1104	16/17
9364	GRC202	0211;68012	3505;4004	0411;09012	11/14
9371	ISH4	0218;1101	1501;4601	04;08	11/13
9368	280599	2604;2402	3901;3802	0901;1501	14/27/28
9367	LCK	0203;1102	38021;4601	0901	14
9394	BPOT	0201	0703;15	1301;15011	10/18/21/27/28
9048	LBUF	3001	1302	07011	12
9032	BSM	0201	1501	0401	11
9237	APA	1101;2403	1502;5502	15011;1405	9/24/25/27/28
9253	THAI742	2403;3303	1512;4601	0406;12021	11/19/20
9369	ISH3	2402	1526N	0406	11
9380	FH6	2402;2901	2702;0705/6	1001;1601	15/29/30
9376	FH4	0101	2703;2705	07;11	12/16/17/18
9266	PAR	11011;2402	2706;4801	15021;11011	16/17/27/28
9377	FH5	2902;0201	2709;4403	1401;07	9/12/24/25
9068	BM9	0201	3501	0801	13
9056	K0SE	0201	3503	1302;1401	9/10/18/21/24/25
9009	KAS011	0101	3701	1601	29/30
9381	FH7	0206;3002	3908;1801	0315;04071	7/8/11
9385	FH11	0301;2902	4404;07021	0101;1101	1/2/4/16/17
9047	PLH	0301	4701	0701	12
9392	GN00218	0301;2902	4703	-	-
9040	BM15	0101	4901	1102	16/17/18
9092	BM92	2501	51011	0404	11
9370	230699	0206;2402	5103;07021	0101;0405	1/2/4/11
9372	ISH5	2402	5401;4801	0901;1405	14/24/25
9052	DBB	0201	5701	0701	12
9267	LE023	6601;3201	7301;51011	1301;1302	9/10/18/21
9382	FH8	11011;3402	8201;27052	07;1503	12/27/28
9366	Daudi	0102;6601	5801;5802	1301;1302	9/10/18/21
9014	MGAR	2601	0801	1501	27/28
9053	HOR	3303	44031	1302	9/10/18/21
9021	RSH	3001;6802	4201	03021	6/7
9024	KT17	0201;1101	15011;3501	0406	11
9016	RML	0204	51011	1602	29/30
9297	HAG	02011	4102	1303	22
9386	FH12	0225;1101	4402;27052	1201;0404	11/19/20
9387	FH13	3402;6802	44031;1501	1503;0301	6/7/8/27/28
9398	FH18	7401;3601	5301;5703	0804;1303	22

HLA-DR Single Box 1.0 Typing Kit Components

- **HLA-DR 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)

- **PCR plates sealers**

  12 plate sealers

- **Instructions manual**

  1 Instructions manual

  [+] With dried specific primers pairs (38 primer pairs and 10 negative controls).

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)** into the PCR mix tube.
6. Vortex the tube vigorously for 15s.
7. Load **10 µl** of the mix into each tube of the plate (**39 primers pairs**).
8. Repeat the previous steps for other DNA sample to complete the HLA-DR 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 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

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

Gel Electrophoresis protocol

PREPARING 2% AGAROSE GEL

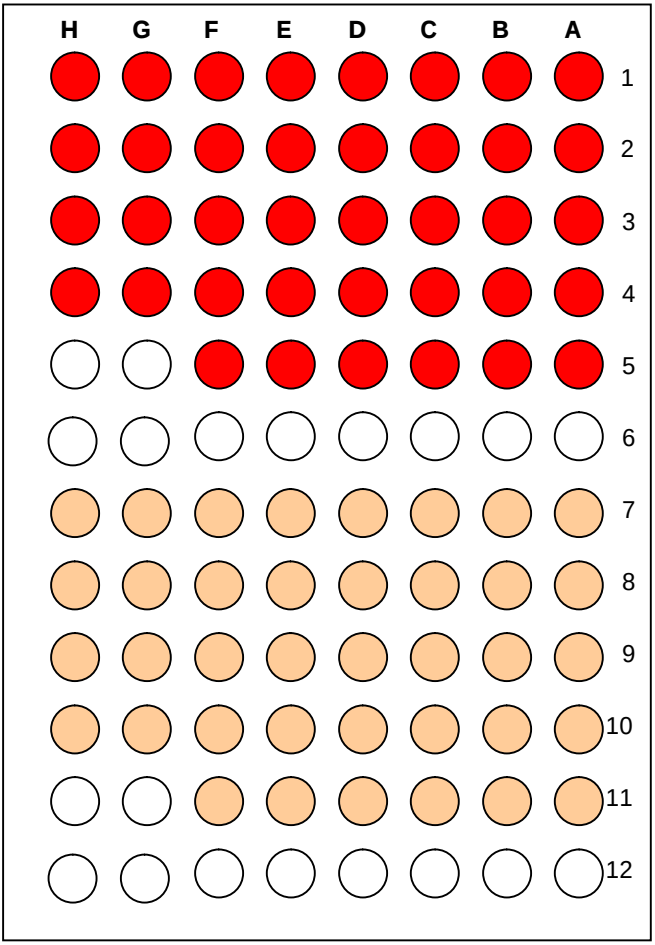
1. Dissolve **4 grams** of electrophoresis **grade agarose** powder in **200 ml** of **1X TAE buffer**.
2. Melt the agarose powder completely in a microwave oven.
3. Cool the heated agarose gel to **~ 50°C.**
4. Add at least 10 µl of **ethidium bromide**[++] (10 mg/ml) or **Sybr Safe**[TM] (100000 x 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-DR Single Box 1.0 plate



HLA-DR Single Box 1.0 Plate Identification

Posição		HLA
1a	7a	DR
1b	7b	DR
1c	7c	DR
1d	7d	DR
1e	7e	DR
1f	7f	DR
1g	7g	DR
1h	7h	DR
2a	8a	DR
2b	8b	DR
2c	8c	DR
2d	8d	DR
2e	8e	DR
2f	8f	DR
2g	8g	DR
2h	8h	DR
3a	9a	DR
3b	9b	DR
3c	9c	DR
3d	9d	DR
3e	9e	DR
3f	9f	DR
3g	9g	DR
3h	9h	DR
4a	10a	DR
4b	10b	DR
4c	10c	DR
4d	10d	DR
4e	10e	DR
4f	10f	DR
4g	10g	DR
4h	10h	DR
5a	11a	DR
5b	11b	DR
5c	11c	DR
5d	11d	DR
5e	11e	DR
5f	11f	DR
5g	11g	Controls
5h	11h	
6a	12a	Empty wells
6b	12b	
6c	12c	
6d	12d	
6e	12e	
6f	12f	
6g	12g	
6h	12h	

Results Interpretation sheet (1/2)

Mix		HLA	Allele	Serotype	Ampl	Contr**
1a	**7a**	DR	DRB1*0101-03/05/06, *1109, *1306	**DRB1*01; 11; 13**	255	790
1b	**7b**	DR	DRB1*0101/021/022/04/05	**DRB1*01**	195	790
1c	**7c**	DR	DRB1*0103	**DRB1*01**	195	790
1d	**7d**	DR	DRB1*0101/03/05, *1109	**DRB1*01; 11**	250	790
1e	**7e**	DR	DRB1*01021, 01022	**DRB1*01**	250	790
1f	**7f**	DR	DRB1*0301-03/05-08/10-13/16	**DRB1*03**	155	790
1g	**7g**	DR	DRB1*0301-10/12-16, *1107	**DRB1*03; 11**	215	790
1h	**7h**	DR	DRB1*0301/04/06/08/10-13/15/16, *1327	**DRB1*03; 13**	220	790
2a	**8a**	DR	DRB1*0302/05/14, *1109/20, *1302/05/26/29/31/34/36/39/41, *1402-03/09/13/19/24/27/30, *1608	**DRB1*03; 11; 13; 14; 16**	190	790
2b	**8b**	DR	DRB1*0301-07/09/11/14-17, *0414, *08042/20, *1301-02/05-11/14-36/18-20/22-25/27-29/34-37/40-42/44, *1402/03/06/09/12/14/17/19-21/23/24/27/29/30/33/36	**DRB1*03; 04; 08; 13; 14**	170	790
2c	**9c**	DR	DRB1 *0401-22/24-33/35/36, *1109/10/21/22, *1306, *1410, *15022	**DRB1*04; 11; 13; 14; 15**	260	790
2d	**8d**	DR	DRB1 *0701, *0703	**DRB1*07**	235	790
2e	**8e**	DR	DRB1 *0801-06/09/10/12/13/16/17/18/22, *1317, *1415	**DRB1*08; 13; 14**	165	790
2f	**8f**	DR	DRB1 *0901	**DRB1*09**	235	790
2g	**8g**	DR	DRB1 *1001	**DRB1*10**	205	790
2h	**8h**	DR	DRB1 *0308, *1101-04/06-21/23-29/32/34/36/37/39/41	**DRB1*11; 03**	175	790
3a	**9a**	DR	DRB1*0308, 1101-21/23-29/32/34/36/37/39/41, *1204, *1411	**DRB1*11; 03; 12; 14**	175	790
3b	**9b**	DR	DRB1 *0414, *1102/03/11/14/16/20/21/36/41, *13011/021/04/08/15-17/19/20/22-24/27-29/31/32/34-36/38-41/43/45/46, *1141, *1204, *1411/16	**DRB1*11; 13; 04; 12; 14**	215	790
3c	**9c**	DR	DRB1 *0812/22, *1201-06 except *1203, *1428	**DRB1*12; 08; 14**	250	790
3d	**9d**	DR	DRB1 *1201-03/05/06	**DRB1*12**	165	790
3e	**9e**	DR	DRB1*1116/20, *1301/02/15/16/27/28/31/32/34-36/39/41	**DRB1*13; 11**	130	790
3f	**9f**	DR	DRB1*0312, *0409, *1303/04/12/13/21/30/32/33/38, *1413	**DRB1*13; 03; 04; 14**	170	790
3g	**9g**	DR	DRB1*1109, *1305/18/26/42, *1427, *1608	**DRB1*11; 13, 14; 16**	130	790
3h	**9h**	DR	DRB1*0809/21, *1117, *1318, *14011/03-05/07/08/10-12/14/15/18/23/26-28/35/36	**DRB1*14; 08; 11, 13**	165	790
DNA 1	**DNA 2**					

Results Interpretation sheet (2/2)

Mix		HLA	Allele	Serotype	Ampl	Contr**
4a	**10a**	DR	DRB1*1117, *14011/04/05/07/08/11/14/18/23/26/28/35/36	**DRB1*14; 11**	255	790
4b	**10b**	DR	DRB1*1109, *1305/06/42,*1402/06/09/13/17/29/30	**DRB1*13; 14; 11**	145	790
4c	**10c**	DR	DRB1*15011-22/03/06/07/08/09	**DRB1*15**	200	790
4d	**10d**	DR	DRB1*1501-09	**DRB1*15**	215	790
4e	**10e**	DR	DRB1*16011/21/03/05/07/08	**DRB1*16**	220	790
4f	**10f**	DR	DRB1*1507, *16011-022/03-05/07/08	**DRB1*16; 15**	215	790
4g	**10g**	DR	DRB5*0202/03, *0106	**DRB5*02, 01**	200	790
4h	**10h**	DR	DRB5*0101/02/05/07/08N/10N	**DRB5*01**	210	790
5a	**11a**	DR	DRB5 all	**DRB5* 01; 02**	210	790
5b	**11b**	DR	DRB3*01011/012/014/03-06	**DRB3*01**	175	790
5c	**11c**	DR	DRB3*0209, *0301-03	**DRB3*03; 02**	175	790
5d	**11d**	DR	DRB1* 0414, *08042, *1306, *1412, *15022, DRB3*0201-06/10/11/13	**DRB3*02** DRB1*04;08;13;14 ;15	175	790
5e	**11e**	DR	DRB1* 0414, *08042, *1306, *1412, *15022, DRB3*0201-06/09-11/13, *0301-03	**DRB3*02; 03** DRB1*04;08;13;14 ;15	175	790
5f	**11f**	DR	DRB4* 0101-05	**DRB4*01**	215	790
5g	**11g**	DQ	Positive control			790
5h	**11h**	DQ	Negative control			
DNA 1	**DNA 2**					

****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 790 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 (1/1)

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
Specific Band	255	195	195	250	250	155	215	220	190	170	260	235	165	235	205	175	175	215	250	165	130	170	130	165	255
DRB1*01	+	+	*	+	*																				
DRB1*03						+	+	+	*	+						*	*					*			
DRB1*04										*	+							*				*			
DRB1*07												+													
DRB1*08										*			+						*					*	
DRB1*09														+											
DRB1*10															+										
DRB1*11									*		*					+	+	*			*		*	*	*
DRB1*12																	*	*	+	+					
DRB1*13									*	+	*		*					+			+	+	+	*	
DRB1*14									+	+	*		*				*	*	*			*	*	+	+
DRB1*15											*														
DRB1*16									*														*		
DRB5*01																									
DRB5*02																									
DRB3*01																									
DRB3*02																									
DRB3*03																									
DRB4*01																									

Well No.	26	27	28	29	30
Specific Band	145	200	215	220	215
DRB1*01					
DRB1*03					
DRB1*04					
DRB1*07					
DRB1*08					
DRB1*09					
DRB1*10					
DRB1*11	*				
DRB1*12					
DRB1*13	*				
DRB1*14	+				
DRB1*15		+	+		*
DRB1*16				+	+
DRB5*01					
DRB5*02					
DRB3*01					
DRB3*02					
DRB3*03					
DRB4*01					

* 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.

- do not pipette by mouth.

Technical Guide

1. DNA Quality and Concentration

For optimal results with the HLA-DR 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-DR Single Box 1.0 Typing Kit™ kits have been intensively tested with the Taq DNA Reagente 5 (Reagente 5, Lisbon, Portugal).

3. PCR Master Mix

For optimal results with the HLA-DR Single Box 1.0 Typing Kit™ the use of the 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. Expiring Date

As specified in the package labels

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-DR 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 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 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-DR Single Box

Product Number: GB.03.06

Intended use: HLA-DR low resolution typing.

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	Nucleotides
	Magnesium chloride Cresol Red	MgCl2
	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.

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

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geneBOX
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