Product Code: 0106

HLA-ABDR 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

biocant – centro de inovação em biotecnologia
núcleo 4, lote 3
3060-197 cantanhede
portugal

tel + 351 231 410 946
fax + 351 231 410 947
e-mail info@genebox.com
www.genebox.com

Table of contents

Presentation

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

Product Changes and Improvements

The HLA-ABDR Box specificity and interpretation tables are constantly updated, to include new HLA 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 Pane*l (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-ABDR low resolution SSP typing kit							
Cell line		Cell Typing			HLA-A	HLA-B	HLA-DR
		HLA-A*	HLA-B*	HLA-DR*	Positive well no.	Positive well no.	Positive well no.
9215	M7	0202;0301	3501;5301	03011;0701	3/4	53/54/55	70/71/72
9273	LADA	0201;8001	0702;5703	09012;1201	3/24	31/39/44	75/79/80
9263	G085	0101;2901	4006;5201	1404;15021	1/17/23	27/28/30/57	84/85/87/88
9373	FH1	0205;6802	1402;5801	1302;0102	3/13/15	37/45	67/68/78/81
9030	JHAF	31012	51011	0407	19/23	27/28/29	72
9035	JBush	3201	3801	1101	20/22	41	77
9045	TUBO	0206;0301	51011	1201;1104	3/4	27/28/29	77/79/80
9220	XLI-ND	0210;3001	1302;4006	0701;09012	3/18	36/57	73/75
9077	T7527	0207	4601	0901;12	3	39/62	75/79/80
9085	EJ32B	3002	1801	03011	18	47	70/71
9103	KT14	2402;2602	4006;51011	09012	6/8/9	27/28/29/57	75
9374	FH2	3401;0201	4001;4402	1302;1301	3/10/26	33/34/57/58/59	78/81
9375	FH3	3301;3101	1402;3502	1101;1104	19/21/23	37/53/54/55	77
9364	GRC202	0211;68012	3505;4004	0411;09012	3/13/14	53/55/57	72/75
9371	ISH4	0218;1101	1501;4601	04;08	3/12	38/39/62	72/74
9368	280599	2604;2402	3901;3802	0901;1501	6/8/9	41/42	75/87/88
9367	LCK	0203;1102	38021;4601	0901	3/12	39/41/62	75
9394	BPOT	0201	0703;15	1301;15011	3	31/38/39	78/81/87/88
9048	LBUF	3001	1302	07011	18	36	73
9032	BSM	0201	1501	0401	3	38/39	72
9237	APA	1101;2403	1502;5502	15011;1405	6/12	39/50	84/85/87/88
9253	THAI742	2403;3303	1512;4601	0406;12021	6/21/23	38/39/62	72/79/80
9369	ISH3	2402	1526N	0406	6	38/39	72
9380	FH6	2402;2901	2702;0705/6	1001;1601	6/17/23	31/52	76/89/90
9376	FH4	0101	2703;2705	07;11	1	52	73/77/78
9266	PAR	11011;2402	2706;4801	15021;11011	6/12	52/59	77/87/88
9377	FH5	2902;0201	2709;4403	1401;07	3/17/23	33/34/52	73/84/85
9068	BM9	0201	3501	0801	3	53/54/55	74
9056	K0SE	0201	3503	1302;1401	3	53/54/55	78/81/84/85
9009	KAS011	0101	3701	1601	1	56	89/90
9381	FH7	0206;3002	3908;1801	0315;04071	3/18	42/47	71/72
9385	FH11	0301;2902	4404;07021	0101;1101	4/17/23	31/33/34	67/68/69
9047	PLH	0301	4701	0701	4	63	73
9392	GN00218	0301;2902	4703	-	4/17/23	63	-
9040	BM15	0101	4901	1102	1	49	77/78
9092	BM92	2501	51011	0404	7/9/25	27/28/29	72
9370	230699	0206;2402	5103;07021	0101;0405	3/6	27/28/29/31	67/68/69/72
9372	ISH5	2402	5401;4801	0901;1405	6	50/59/66	75/84/85
9052	DBB	0201	5701	0701	3	44	73
9267	LE023	6601;3201	7301;51011	1301;1302	9/10/20/22/25	27/28/29/65	78/81
9382	FH8	11011;3402	8201;27052	07;1503	12/14/26	50/52	73/87/88
9366	Daudi	0102;6601	5801;5802	1301;1302	1/9/10/25	45	78/81
9014	MGAR	2601	0801	1501	8/25	32	87/88
9053	HOR	3303	44031	1302	21/23	33/34	78/81
9021	RSH	3001;6802	4201	03021	13/15/18	61	70/71
9024	KT17	0201;1101	15011;3501	0406	3/12	38/39/53/54/55	72
9016	RML	0204	51011	1602	3	27/28/29	89/90
9297	HAG	02011	4102	1303	3	59	82
9386	FH12	0225;1101	4402;27052	1201;0404	3/12	33/34/52	72/79/80
9387	FH13	3402;6802	44031;1501	1503;0301	9/10/13/15/26	33/34/38/39	70/71/87/88
9398	FH18	7401;3601	5301;5703	0804;1303	2/22/23	46/44/54	74/82

HLA-ABDR Box 1.0 Typing Kit Components

- **HLA-ABDR typing plate**[+] (12 typings)

  12 plates (one sample each plate) (Keep at -30/ -15 ºC)

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

  12 X 310 µl (keep at -15 / -30ºC)

- **PCR sealers**

  12 PCR 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 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:
   - **305 µl of PCR Master Mix,**
   - **615 µl of ddH_2O, and**
   - **80 µl of DNA sample (conc. 100-200 ng / µl)**

   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 each tube of the plate (**96 primers pairs**).
5. 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

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

Gel Electrophoresis protocol

PREPARING 2% AGAROSE GEL

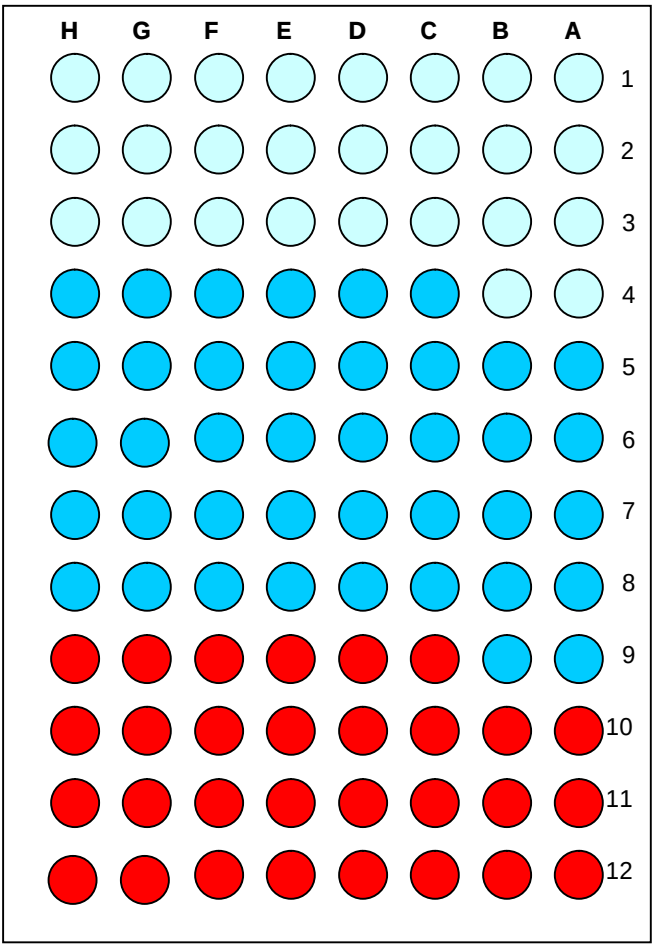
1. Dissolve **4 grams** of electrophoresis **grade agarose** powder in **200 ml** of **1X TAE buffer**.
2. Melt the agarose powder completely in a microwave oven.
3. Cool the heated agarose gel to **~ 50°C.**
4. Add at least 10 µl of **ethidium bromide**[++] (10 mg/ml) or **Sybr Safe**[TM] (100000 x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

HLA-ABDR Box 1.0 plate



HLA-ABDR Box 1.0 Plate Identification

Position	HLA	Position	HLA
1a	A	7a	B
1b	A	7b	B
1c	A	7c	B
1d	A	7d	B
1e	A	7e	B
1f	A	7f	B
1g	A	7g	B
1h	A	7h	B
2a	A	8a	B
2b	A	8b	B
2c	A	8c	B
2d	A	8d	B
2e	A	8e	B
2f	A	8f	B
2g	A	8g	B
2h	A	8h	B
3a	A	9a	B
3b	A	9b	B
3c	A	9c	DR
3d	A	9d	DR
3e	A	9e	DR
3f	A	9f	DR
3g	A	9g	DR
3h	A	9h	DR
4a	A	10a	DR
4b	A	10b	DR
4c	B	10c	DR
4d	B	10d	DR
4e	B	10e	DR
4f	B	10f	DR
4g	B	10g	DR
4h	B	10h	DR
5a	B	11a	DR
5b	B	11b	DR
5c	B	11c	DR
5d	B	11d	DR
5e	B	11e	DR
5f	B	11f	DR
5g	B	11g	DR
5h	B	11h	DR
6a	B	12a	DR
6b	B	12b	DR
6c	B	12c	DR
6d	B	12d	DR
6e	B	12e	DR
6f	B	12f	DR
6g	B	12g	DR
6h	B	12h	DR

Results Interpretation sheet (1/4)

Well	HLA	Allele	Serotype	ampl	cont**
1	A	A*0101-08	**A*01**	574	256
2	A	A*3601/02	**A*36**	563	256
3	A	A*02	**A*02**	813	256
4	A	A*0301-04/08, 2418, 3204, 3602, 6819	**A*03; 24; 32; 36; 68**	626	256
5	A	A*2301-06, 0246, 2413, 2416, 2418	**A*23; 02; 24;**	555	256
6	A	A*2402-30 except *2406/8/13/16/18/22/24/25	**A*24**	555	256
7	A	A*2501-03	**A*25**	398	256
8	A	A*2601/02/04/07-15,*4301	**A*26; 43**	400	256
9	A	A*2501-03,*2601-15 except 2607,*3401-03,*6601-3	**A*25; 26**	438	256
10	A	A*3401-03,*6601/2,*2502,*2613	**A*34; 66; 25; 26**	417	256
11	A	A*4301	**A*43**	440	256
12	A	A*1101/02/04-07/09	**A*11**	518	256
13	A	A*6801/2,*6901/06-14/16-19,*0234/35,*2407/24,	**A*68; 69; 02; 24**	445	256
14	A	A*6801/03/04/05/08/09/11/12/16/19	**A*68**	426	256
15	A	A*6802/15/18N	**A*68**	623	256
16	A	A*6901,*0234/35	**A*69; 02**	405	256
17	A	A*2901-04	**A*29**	515	256
18	A	A*3001-09	**A*30**	560	256
19	A	A*3101/02/05	**A*31**	481	256
20	A	A*3201/02/05/06	**A*32**	421	256
21	A	A*3301/03-06	**A*33**	461	256
22	A	A*3201-03/05/06,*7401-04, B*4405	**A*32; 74; B*44**	494	256
23	A	A*2901-04,*3101/02/05,*3301/03-06,*7401/02/04	**A*29; 31; 33; 74**	414	256
24	A	A*8001	**A*80**	543	256
25	A	A*2501, *2601/3/5/7/8,*6601/2/3, *4301 *6602/03	**A*25; 26; 66; 43; 66**	170	256
26	A	A*3401-03,*2609,*3103/04	**A* 34; 26; 31**	170	256

Results Interpretation sheet (2/4)

Well	HLA	Allele	Serotype	ampl	cont**
27	B	B*5101-23 except *5115,*5201/03,*7801-04	**B*51; 52; 78**	504	256
28	B	B*5101-23 except *5110/131/15,*5201-03	**B*51; 52**	401	256
29	B	B*5101-23 except *5110/131/15,*7801/2,*1509	**B*51; 78; 15**	451	256
30	B	B*5201-03,*15012,*4026,*4028,*5107	**B*52; 15; 40; 51**	440	256
31	B	B*0702-24 except *0713/19/21,*4025, *8101,*3907,*4806, *6812	**B*07; 40; 81; 39; 48; 68**	619	256
32	B	B*0801-03/06-12,*3510	**B*08; 35**	606	256
33	B	B*4402-22 except *4406/06/15/16	**B*44**	537	256
34	B	B*4402-22 except 4406/16,*4501-03,*1514, *5002	**B*44; 45; 15; 50**	575	256
35	B	B*4501/03,*4901-03,*5001/02/04,*5402	**B*45; 49; 50; 54**	600	256
36	B	B*1301/02/06	**B*13**	471	256
37	B	B*1401-04	**B*14**	390	256
38	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,*4802, *1304,*1801-12,*3520/28,*4003/20,*4417	**B*15; 48; 13; 18; 35; 40; 44**	422	256
39	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
40	B	B*1509/10/18/21/23/27/51,*3526,*5122,*7803	**B*15; 35; 51; 78**	562	256
41	B	B*3801-06,*5119,*3920	**B*38; 51; 39**	500	256
42	B	B*1548,*3535,*39011-19/22-24,*6701	**B*15; 35; 39; 67**	507	256
43	B	B*67011/67012,*3910-17/20	**B*67; 39**	548	256
44	B	B*5701-14 except *5705	**B*57**	380	256
45	B	B*5801/04/05,*5705	**B*58; 57**	345	256
46	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
47	B	B*1801-12	**B*18**	503	256
48	B	B*4901/03,*5901,*5115,*4418	**B*49; 59; 51; 44**	385	256
49	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
50	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
51	B	B*5601-07,*8201/02,*8301,*0720/24	**B*56; 82; 83; 07**	551	256
52	B	B*3501-37 except *3512/20/22/26/28/31,*5301-06	**B*35; 53**	390	256
53	B	B*3501-4/6-9/11/12,*5301,*5104, *1502,*1513*1521,*4406	**B*35; 15; 44**	369	256
54	B	B*2701-22 except *2712/16/18	**B*27**	150	256

Results Interpretation sheet (3/4)

Well	HLA	Allele	Serotype	ampl	cont**
55	B	B*3501-37 except *3501/19/25-27,*18,*7801-04,*1522/59,*5305,*5606	**B*35; 18; 78; 15; 53; 56**	128	256
56	B	B*3701,*4406,*5108	**B*37; 44; 51**	606	256
57	B	B*4001-34 except *4012/14/17/21/28,*4701-03	**B*40; 47**	544	256
58	B	B*4001/07/14-16/22N/23/25/30-34,*27053,*3907	**B* 40; 27; 39**	784	256
59	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
60	B	B*4101-05,*4202,*4405/18,*4501-03,*5002,*27053,*3907	**B*41; 42; 44; 45; 50; 27; 39**	781	256
61	B	B*4201/02,*0801-12,*3510,*3907	**B*42; 08; 35; 39**	702	256
62	B	B*4601/02	**B*46**	459	256
63	B	B*4701-03,*2718,*3702	**B*47; 37; 27**	414	256
64	B	B*5401/02,	**B*54**	508	256
65	B	B*7301	**B*73**	289	256
66	B	B*7801-04, *1509/012,*4026/28,*5605/06	**B*78; 15; 40; 56**	400	256
Well	**HLA**	**Allele**	**Serotype**	**ampl**	**cont****
67	DR	DRB1*0101-03/05/06, *1109, *1306	**DRB1*01; 11; 13**	255	790
68	DR	DRB1*0101/021/022/04/05	**DRB1*01**	195	790
69	DR	DRB1*0101/03/05, *1109	**DRB1*01; 11**	250	790
70	DR	DRB1*0301-03/05-08/10-13/16	**DRB1*03**	155	790
71	DR	DRB1*0301-10/12-16, *1107	**DRB1*03; 11**	215	790
72	DR	DRB1 *0401-22/24-33/35/36, *1109/10/21/22, *1306, *1410, *15022	**DRB1*04; 11; 13; 14; 15**	260	790
73	DR	DRB1 *0701, *0703	**DRB1*07**	235	790
74	DR	DRB1 *0801-06/09/10/12/13/16/17/18/22, *1317, *1415	**DRB1*08; 13; 14**	165	790
75	DR	DRB1 *0901	**DRB1*09**	235	790
76	DR	DRB1 *1001	**DRB1*10**	205	790
77	DR	DRB1 *0308, *1101-04/06-21/23-29/32/34/36/37/39/41	**DRB1*03; 11**	175	790
78	DR	DRB1 *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, *0414, *1204, *1411/16	**DRB1*11; 13; 04; 12; 14**	215	790
79	DR	DRB1 *1201-06 except *1203, *0812/22, *1428	**DRB1*12; 08; 14**	250	790

Results Interpretation sheet (4/4)

Well	HLA	Allele	Serotype	ampl	cont**
80	DR	DRB1*1201-03/05/06	**DRB1*12**	165	790
81	DR	DRB1*1301/02/15/16/27/28/31/32/34-36/39/41; *1116/20	**DRB1*13; 11**	130	790
82	DR	DRB1*1303/04/12/13/21/30/32/33/38, *0312, *0409, *1413	**DRB1*13; 03; 04; 14**	170	790
83	DR	DRB1*1109, *1305/18/26/42, *1427, *1608	**DRB1*11; 13, 14; 16**	130	790
84	DR	DRB1*14011/03-05/07/08/10-12/14/15/18/23/26-28/35/36, *0809/21, *1117, *1318	**DRB1*14; 08; 11, 13**	165	790
85	DR	DRB1*14011/04/05/07/08/11/14/18/23/26/28/35/36, *1117	**DRB1*14; 11**	255	790
86	DR	DRB1*1305/06/42,*1402/06/09/13/17/29/30,*1109	**DRB1*13; 14; 11**	145	790
87	DR	DRB1*15011-22/03/06/07/08/09	**DRB1*15**	200	790
88	DR	DRB1*1501-09	**DRB1*15**	215	790
89	DR	DRB1*16011/21/03/05/07/08	**DRB1*16**	220	790
90	DR	DRB1*16011-022/03-05/07/08, *1507	**DRB1*16; 15**	215	790
Well	**HLA**	**Allele**	**Serotype**	**ampl**	**cont****
91	DR	DRB5*0202/03, *0106	**DRB5*02, 01**	200	790
92	DR	DRB5*0101/02/05/07/08N/10N	**DRB5*01**	210	790
93	DR	DRB3*01011/012/014/03-06	**DRB3*01**	175	790
94	DR	DRB3*0301-03, *0209	**DRB3*03; 02**	175	790
95	DR	DRB3*0201-06/10/11/13 DRB1* 0414, *08042, *1306, *1412, *15022	**DRB3*02** DRB1*04;08;13;14;15	175	790
96	DR	DRB4* 0101-05	**DRB4*01**	215	790

DNA

****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 (1/2)

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
Specific Band	574	563	813	626	555	555	398	400	438	417	440	518	445	426	623	405	515	560	481	421	461	494	414	543	170	170
A*01	+																									
A*02			+																							
A*03				+																						
A*11												+														
A*23					+																					
A*24						+																				
A*25							+		+																+	
A*26								+	+																+	
A*29																	+						+			
A*30																		+								
A*31																			+							
A*32																				+						
A*33																					+					
A*34									+	+				+												+
A*36		+																								
A*43								+			+														+	
A*66									+	+															+	
A*68													+	+	+											
A*69													+			+										
A*74																						+	+			
A*80																								+		

Well No.	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
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.	55	56	57	58	59	60	61	62	63	64	65	66
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												

Well No.	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	81	88	89	90	91	92	93	94	95	96
Specific Band	255	195	250	155	215	260	235	165	235	205	175	215	250	165	130	170	130	165	255	145	200	215	220	215	200	210	175	175	175	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-ABDR 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-ABDR 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-ABDR 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. 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-ABDR 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-ABDR Box

Product Number: GB.01.06

Intended use: HLA-A, HLA-B, and HLA-C 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

4. Olerup O, Zetterquist H.HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation.*Tissue Antigens*. 1992; 39: 225-35.

CE 0197

geneBOX
DESENVOLVIMENTO E PRODUÇÃO DE TESTES DE DIAGNÓSTICO

biocant – centro de inovação em biotecnologia
núcleo 4, lote 3
3060-197 cantanhede
portugal

tel + 351 231 410 946
fax + 351 231 410 947
e-mail info@genebox.com
www.genebox.com