

Product Code: 0205

HLA-ABC Box 1.0 Typing Kit

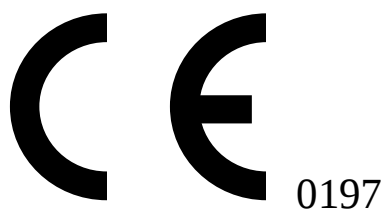
In vitro diagnostics disposal

Instructions Manual



DESENVOLVIMENTO E PRODUÇÃO
DE TESTES DE DIAGNÓSTICO

Version 1.8; 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-A, HLA-B and HLA-C genes.

Product Changes and Improvements

The HLA-ABC 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

HLA-ABC low resolution SSP typing kit							
Cell line		Cell Typing			HLA-A Positive well no.	HLA-B Positive well no.	HLA-Cw Positive well no.
		HLA-A*	HLA-B*	HLA-Cw*			
9215	M7	0202;0301	3501;5301	0401	3/4/25/36	55/56/57/69/70	76
9273	LADA	0201;8001	0702;5703	0701;0802	3/24/25/26	33/41/46/69/70	80/84
9263	G085	0101;2901	4006;5201	12022;1502	1/17/23/26	29/30/32/59/69/70	85/86/89
9373	FH1	0205;6802	1402;5801	0701;0802	3/13/15/25	39/47/69/70	80/84
9030	JHAF	31012	51011	1502	19/23/26	29/30/31/69	89
9035	JBush	3201	3801	1203	20/22/26	43/69	87
9045	TUBO	0206;0301	51011	0704;1502	3/4/25/26	29/30/31/69	83/89
9220	XLI-ND	0210;3001	1302;4006	0602;0801	3/18/25/26	38/59/69/70	78/84
9077	T7527	0207	4601	0102	3/25	41/64/70	71
9085	EJ32B	3002	1801	0501	18/26	49/70	77
9103	KT14	2402;2602	4006;51011	0801;1402	6/8/9/25/26	29/30/31/59/69/70	84/88
9374	FH2	3401;0201	4001;4402	0501;0304	3/10/25/26/28	35/36/59/60/61/69/70	77/74
9375	FH3	3301;3101	1402;3502	0401;0802	19/21/23/26	39/55/56/57/70	76/84
9364	GRC202	0211;68012	3505;4004	03041;04011	3/13/14/25	55/57/59/70	74/76
9371	ISH4	0218;1101	1501;4601	04011;0102	3/12/25/26	40/41/64/70	71/76
9368	280599	2604;2402	3901;3802	0702/3	6/8/9/25/26	43/44/69/70	81
9367	LCK	0203;1102	38021;4601	0702;1202	3/12/25/26	41/43/64/69/70	81/85/86
9394	BPOT	0201	0703;15	03031	3/25	33/40/41/70	73
9048	LBUF	3001	1302	0602	18/26	38/69	78
9032	BSM	0201	1501	0304	3/25	40/41/70	74
9237	APA	1101;2403	1502;5502	0801;1203	6/12/25/26	41/52/70	84/87
9253	THAI742	2403;3303	1512;4601	0102;03031	6/21/23/25/26	40/41/64/70	71/73
9369	ISH3	2402	1526N	04011	6/25	40/41/70	76
9380	FH6	2402;2901	2702;0705/6	02022;1505	6/17/23/25/26	33/54/69/70	79/92
9376	FH4	0101	2703;2705	02022	1/26	54/69	72
9266	PAR	11011;2402	2706;4801	03041;0801	6/12/26/25	54/61/69/70	74/84
9377	FH5	2902;0201	2709;4403	0102;1601	3/17/23/25/26	35/36/54/69	71/93
9068	BM9	0201	3501	0401	3/25	55/56/57/70	76
9056	K05E	0201	3503	1203	3/25	55/56/57/70	87
9009	KAS011	0101	3701	0602	1/26	58/69	78
9381	FH7	0206;3002	3908;1801	0702;0501	3/18/25/26	44/49/70	77/81
9385	FH11	0301;2902	4404;07021	0702;1601	4/17/23/26	33/35/36/69	81/93
9047	PLH	0301	4701	0602	4/26	65/69	78
9392	GN00218	0301;2902	4703	0602;0701	4/17/23/26	65/69	78/80
9040	BM15	0101	4901	0701	1/26	51/69	80
9092	BM92	2501	51011	0102	7/9/26/27	29/30/31/69	71
9370	230699	0206;2402	5103;07021	0702;1402	3/6/25	29/30/31/33/69/70	81/88
9372	ISH5	2402	5401;4801	0803;0102	6/25	52/61/66/70	71/84
9052	DBB	0201	5701	0602	3/25	46/69	78
9267	LE023	6601;3201	7301;51011	0102;15051	9/10/20/22/26/27	29/30/31/67/70	71/92
9382	FH8	11011;3402	8201;27052	0302;0102	12/14/26/28	52/54/69/70	71/74/75
9366	Daudi	0102;6601	5801;5802	0302;0602	1/9/10/26/27	47/69	74/75/78
9014	MGAR	2601	0801	0701	8/26/27	34/70	80
9053	HOR	3303	44031	1403	21/23/26	35/36/69	88
9021	RSH	3001;6802	4201	1701	13/15/18/25/26	63/70	95
9024	KT17	0201;1101	15011;3501	0303;0401	3/12/25/26	40/41/55/56/57/70	73/76
9016	RML	0204	51011	1502	3/25	29/30/31/69	89
9297	HAG	02011	4102	1701	3/25	61/70	95
9386	FH12	0225;1101	4402;27052	0501;02022	3/12/25/26	35/36/54/69	72/77
9387	FH13	3402;6802	44031;1501	0401;03042	9/10/13/15/25/26/28	35/36/40/41/69/70	76/74
9398	FH18	7401;3601	5301;5703	0401;0701	2/22/23/26	48/46/56/69	76/80

HLA-ABC Box 1.0 Typing Kit Components

- **HLA-ABC low resolution typing plate (12 typings)⁺**

12 plates (1 sample each) (Keep at -30 / -15 °C)

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

12 X 310 µl (keep at -15 / -30°C)

- **Plate sealers**

12 PCR sealers

- **MaInstructions manual**

1 Instructions Manual

⁺ With dried specific primers pares.

PCR Master Mix Components

Nucleotides

Final concentration of each dNTP: 600 µM

PCR Buffer

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

Glycerol

Final concentration: 16,6%

Cresol Red

Final concentration: 300µg/ml

PCR amplification protocol

Reagents

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

DNA Extraction

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

Alternatively, the DNA can be extracted using trimethylammonium-bromide salts (DTAB/CTAB) or by salting out, dissolving it in dH₂O or 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 ddH₂O**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 **80 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 (**95 primers pairs**).

7. Repeat the previous steps for other DNA sample to complete the HLA-ABC 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 °C	1 min	1
Denaturation	96 °C	25 sec	5
Annealing	70 °C	45 sec	
Extension	72 °C	30 sec	
Denaturation	96 °C	25 sec	21
Annealing	65 °C	45 sec	
Extension	72 °C	30 sec	
Denaturation	96 °C	25 sec	4
Annealing	55 °C	1 min	
Extension	72 °C	2 min	
Extension	72 °C	10 min	1
Keep (optional)	4 °C	Infinite	1

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

Gel Electrophoresis protocol

PREPARING 2 % AGAROSE GEL

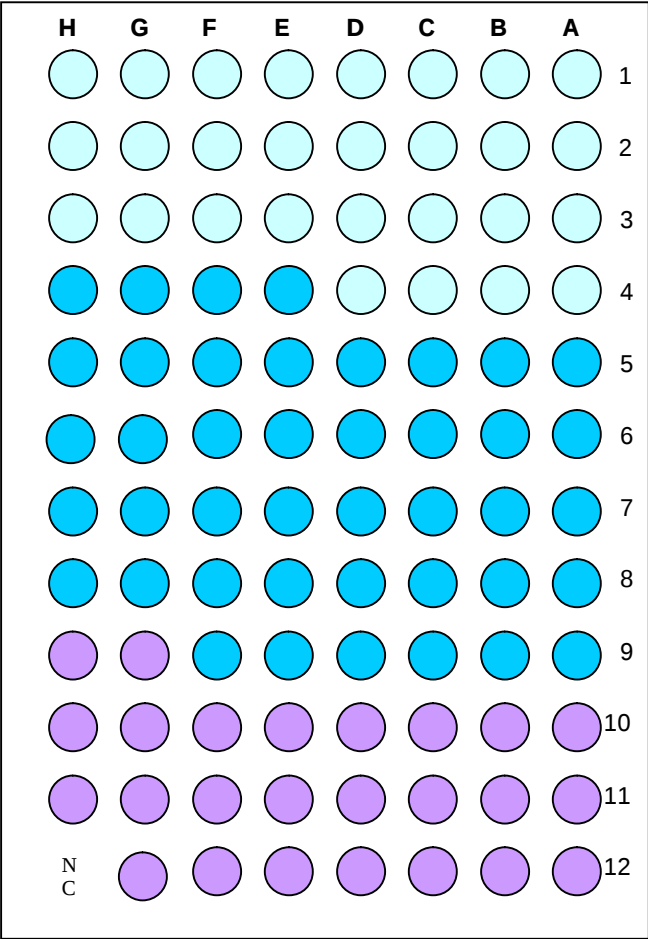
1. Dissolve **4 grams** of electrophoresis **grade agarose** powder in **200 ml** of **1X TAE buffer**.
2. Melt the agarose powder completely in a microwave oven.
3. Cool the heated agarose gel to **50°C**.
4. Add at least 10 µl of **ethidium bromide⁺⁺** (10 mg/ml) or **Sybr SafeTM** (100000 x concentrate)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

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

GEL ELECTROPHORESIS

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

HLA-ABC Box 1.0 plate



HLA-ABC 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	B
3d	A	9d	B
3e	A	9e	B
3f	A	9f	B
3g	A	9g	C
3h	A	9h	C
4a	A	10a	C
4b	A	10b	C
4c	A	10c	C
4d	A	10d	C
4e	B	10e	C
4f	B	10f	C
4g	B	10g	C
4h	B	10h	C
5a	B	11a	C
5b	B	11b	C
5c	B	11c	C
5d	B	11d	C
5e	B	11e	C
5f	B	11f	C
5g	B	11g	C
5h	B	11h	C
6a	B	12a	C
6b	B	12b	C
6c	B	12c	C
6d	B	12d	C
6e	B	12e	C
6f	B	12f	C
6g	B	12g	C
6h	B	12h	Negative Control

Results Interpretation sheet (1/4)

Mix	HLA	Allele	Serotype	ampl	Contr**
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*0201-46 except *0223/24,*2301/02/04-06,*2402-30 except *2416/19/21,*6801-19,*6901	A*02; 24; 68; 69	446	256
26	A	All except A*0201-46 except 23/24,*2301/02/04-06,*2402-30 except 16/19/21,*6801-19,*6901	A*01; 03; 11; 23; 25; 26; 29; 30; 31; 32; 33; 34; 36; 43; 66; 74; 80	446	256
27	A	A*2501, *2601/3/5/7/8,*6601/2/3,*4301 *6602/03	A*25; 26; 66; 43; 66	170	256
28	A	A*3401-03,*2609,*3103/04	A* 34; 26; 31	170	256

Results Interpretation sheet (2/4)

Mix	HLA	Allele	Serotype	ampl	Contr**
29	B	B*5101-23 except *5115,*5201/03,*7801-04	B*51; 52; 78	504	256
30	B	B*5101-23 except *5110/131/15,*5201-03	B*51; 52	401	256
31	B	B*5101-23 except *5110/131/15,*7801/2,*1509	B*51; 78; 15	451	256
32	B	B*5201-03,*15012,*4026,*4028,*5107	B*52; 15; 40; 51	440	256
33	B	B*0702-24 except *0713/19/21,*4025,*8101,*3907,*4806,*6812	B*07; 40; 81; 39; 48; 68	619	256
34	B	B*0801-03/06-12,*3510	B*08; 35	606	256
35	B	B*4402-22 except *4406/06/15/16	B*44	537	256
36	B	B*4402-22 except 4406/16,*4501-03,*1514,*5002	B*44; 45; 15; 50	575	256
37	B	B*4501/03,*4901-03,*5001/02/04,*5402	B*45; 49; 50; 54	600	256
38	B	B*1301/02/06	B*13	471	256
39	B	B*1401-04	B*14	390	256
40	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
41	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
42	B	B*1509/10/18/21/23/27/51,*3526,*5122,*7803	B*15; 35; 51; 78	562	256
43	B	B*3801-06,*5119,*3920	B*38; 51; 39	500	256
44	B	B*1548,*3535,*39011-19/22-24,*6701	B*15; 35; 39; 67	507	256
45	B	B*67011/67012,*3910-17/20	B*67; 39	548	256
46	B	B*5701-07 except *5705	B*57	380	256
47	B	B*5801/04/05,*5705	B*58; 57	345	256
48	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
49	B	B*1801-12	B*18	503	256
50	B	B*4901/03,*5901,*5115,*4418	B*49; 59; 51; 44	385	256
51	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
52	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
53	B	B*5601-07,*8201/02,*8301,*0720/24	B*56; 82; 83; 07	551	256
54	B	B*2701-22 except *2712/16/18	B*27	150	256
55	B	B*3501-37 except *3512/20/22/26/28/31,*5301-06	B*35; 53	390	256
56	B	B*3501-04/06-09/11/12/14/15/17-21/23-27/29/30/33-36,*0712/14/18,*1502/13/21/24,*4406/12,*5301-06,*5104*8301	B*35; 07; 15; 44; 51; 53; 83	369	256

Results Interpretation sheet (3/4)

Mix	HLA	Allele	Serotype	ampl	Contr**
57	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
58	B	B*3701,*4406,*5108	B*37; 44; 51	606	256
59	B	B*4001-34 except *4012/14/17/21/28,*4701-03	B*40; 47	544	256
60	B	B*4001/07/14-16/22N/23/25/30-34,*27053,*3907	B* 40; 27; 39	784	256
61	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
62	B	B*4101-05,*4202,*4405/18,*4501-03,*5002,*27053,*3907	B*41; 42; 44; 45; 50; 27; 39	781	256
63	B	B*4201/02,*0801-12,*3510,*3907	B*42; 08; 35; 39	702	256
64	B	B*4601/02	B*46	459	256
65	B	B*4701-03,*2718,*3702	B*47; 37; 27	414	256
66	B	B*5401/02,	B*54	508	256
67	B	B*7301	B*73	289	256
68	B	B*7801-04, *1509/012,*4026/28,*5605/06	B*78; 15; 40; 56	400	256
69	B	Bw4	B*08; 13; 15; 18; 27; 37; 38; 44; 47; 49; 51; 52; 53; 57; 58; 59	180	256
70	B	Bw6 not 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

Results Interpretation sheet (4/4)

Mix	HLA	Allele	Serotype	ampl	Contr**
71	Cw	Cw*0102	Cw*01	1026	1600+796
72	Cw	Cw*0202,*1701/2	Cw*02; 17	521	1600+796
73	Cw	Cw*0303	Cw*03	530	1600+796
74	Cw	Cw*0302/4	Cw*03	529	1600+796
75	Cw	Cw*0302	Cw*03	206	1600+796
76	Cw	Cw*0401-3, *1801	Cw*04; 18	330	1600+796
77	Cw	Cw*0501	Cw*05	563	1600+796
78	Cw	Cw*0602	Cw*06	304	1600+796
79	Cw	Cw*1801	Cw*18	500	1600+796
80	Cw	Cw*0701	Cw*07	516	1600+796
81	Cw	Cw*0702/3	Cw*07	302	1600+796
82	Cw	Cw*0703, A*2604	Cw*07 A*26	494	1600+796
83	Cw	Cw*0704	Cw*07	563	1600+796
84	Cw	single 162bp=Cw*0802,162&632bp=Cw*0801/3	Cw*08		1600+796
85	Cw	Cw*1202,*1301	Cw*12; 13	449	1600+796
86	Cw	Cw*1202	Cw*12	537	1600+796
87	Cw	Cw*1203	Cw*12	453	1600+796
88	Cw	Cw*1402/3	Cw*14	541	1600+796
89	Cw	Cw*1502	Cw*15	407	1600+796
90	Cw	Cw*1503	Cw*15	406	1600+796
91	Cw	Cw*1504,*0701	Cw*15; 07	423	1600+796
92	Cw	Cw*1505	Cw*15	512	1600+796
93	Cw	Cw*1601	Cw*16	424	1600+796
94	Cw	Cw*1602	Cw*16	475	1600+796
95	Cw	Cw*1701/2	Cw*17	512	1600+796
96	Cw	Negative control			

****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	574	563	813	626	555	555	388	440	443	447	441	548	445	446	643	445	547	548	449	442	441	444	443	544	446	444	147	148
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.	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	55	56
Specific Band	594	401	411	423	633	644	503	567	570	678	431	432	433	434	500	507	548	346	347	348	549	350	651	452	253	154	355	356
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.	5 7	5 8	5 9	6 0	6 1	6 2	6 3	6 4	6 5	6 6	6 7	6 8
Specific Band	1 2 8	6 0 6	5 4 4	7 8 4	6 8 8	7 0 1	7 0 2	4 5 9	4 1 4	5 0 8	2 8 9	4 0 0
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.	7 1	7 2	7 3	7 4	7 5	7 6	7 7	7 8	7 9	8 0	8 1	8 2	8 3	8 4	8 5	8 6	8 7	8 8	8 9	9 0	9 1	9 2	9 3	9 4	9 5
Specific Band	1 0 2 6	5 2 1	5 3 0	5 2 9	2 0 6	3 3 0	5 6 3	3 0 4	5 0 0	5 1 6	3 0 2	4 9 4	5 6 3	1 6 2	4 4 9	5 3 7	4 5 3	5 4 1	4 0 7	4 0 6	4 2 3	4 2 4	5 1 2	5 1 2	4 7 5
Cw*01	+																								
Cw*02		+																							
Cw*03			*	*	*																				
Cw*04						+																			
Cw*05							+																		
Cw*06								+																	
Cw*07										+	*	*	*								*				
Cw*08						+								+											
Cw*12																+	*	*							
Cw*13																+									
Cw*14																		+							
Cw*15																			*	*	*	*			
Cw*16																							*	*	
Cw*17		+																							+
Cw*18									+																
A*2604												+													

* 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 ddH_2O in order to have the proper concentration Repeat typing with a good quality DNA sample
	Contamination with previously amplified PCR products or with other DNA samples during the DNA extraction or PCR preparation steps	Clean the working area Work in separated pre-PCR and post-PCR rooms Keep different lab coats in pre-PCR and post-PCR rooms Change protective gloves frequently Repeat typing with a good quality DNA sample
Blurred bands	Degradation of DNA sample	Re-extract the DNA sample with fresh material Repeat typing with a good quality DNA sample
	Excess of template DNA	Check DNA quality and concentration Dissolve the DNA sample in ddH_2O in order to have the proper concentration Repeat typing with a good quality DNA sample
	Electrophoresis Buffer Problems: wrong buffer or older buffer	Use a fresh recommended buffer

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

6. 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-ABC Box typing kit have the specificities given in the Results Interpretations Sheet/Tables of the product insert.

1. Typing plate

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

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

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

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

2. PCR Master Mix

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

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

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

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

3. DNA

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

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

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

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-ABC Box

Product Number: GB.02.05

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

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 MgCl ₂ 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 25°C.
Transportation should not exceed 3 days.

15. Other information

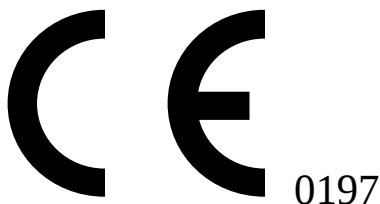
The above information is based on our current level of knowledge, but does not purport to be all-inclusive and shall be used only as a guide. *geneBOX - R&D Diagnostic Tests* shall not be held liable for any damage resulting from handling or from contact with the above products.

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

+ 351 231 410 946

References

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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. Schaffer M, Olerup O. HLA-AB typing by polymerase-chain reaction with sequence-specific primers: more accurate, less errors, and increased resolution compared to serological typing. *Tissue Antigens*. 2001; 58: 299-307.



DESENVOLVIMENTO E PRODUÇÃO
DE TESTES DE DIAGNÓSTICO

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