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Manual No.: 12
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Lot No.: DR11L04-4

CTS *Collaborative Transplant Study*



WORKING INSTRUCTION

HLA-DRB1*11 SUBTYPING CTS-PCR-SSP MINITRAYS

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following products:

Product No.	Description
212	HLA-DRB1*11 Subtyping CTS-PCR-SSP Minitray Kit

  **0197**

CHANGES COMPARED TO LOT DR11L04-3:

This kit was updated with the IMGT/HLA Sequence Database Release 2.24.0, January 2009. No changes in the total number of mixes. Except mix 9, 15, 21 and 23 - 25, the specificities of all remaining mixes have been extended by new HLA-alleles.

Introduction

- Intended use: This kit reveals a high resolution typing of HLA-DRB1*11 by the PCR-SSP method.
- Allele coverage: IMGT/HLA Sequence Database Release 2.24.0, January 2009.
- This manual is only valid for **Lot No. DR11L04-4**.
- This manual should be used together with the Main Manual (General Information) the 'Working Instruction for the CTS-PCR-SSP **TRAY and MINITRAY KITS**' (Manual No. 100A).

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1. Kit Composition

MINITRAY KIT

- Number of PCR primer mixes per test: 30 (29 allele-specific and 1 negative control mix)
- Number of tests per kit: 12
- The primer mixes are aliquoted and lyophilized in thin-walled, green PCR-minitrays.
- PCR buffer: 1.3 ml of 5% Mastermix (without Taq polymerase)

For storage condition, please refer to Section 1 of the ‘Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS’ (Manual No. 100A) supplied along with this product.

2. Materials, Reagents and Equipment not supplied

Please refer to Section 2 of the ‘Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS’ (Manual No. 100A) supplied along with this product.

3. Sample Requirements, PCR and Gel Electrophoresis

Please refer to Section 3 to 6 of the ‘Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS’ (Manual No. 100A) supplied along with this product.

4. Result Evaluation

- 4.1 Check the approximate size of the PCR product against the Primer Mix Specificity Table (Table 1) to confirm the correct product size.
- 4.2 Use the Amplification Pattern Tables (Table 2 and 3) to make allele assignment. Alternatively, you can use the SCORE Software (www.IHWG.org) for detailed result interpretation.

5. Interpretation Hints

The quality and quantity of DNA as well as of the Taq polymerase are extremely crucial factors. If your bands are too weak, you might try to adjust these two factors until you obtain optimal results.

Please refer to Section 7 of the 'Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No. 100A) supplied along with this product

SPECIAL NOTES

- Some other HLA-DRB alleles share specific sequence motives with HLA-DRB1*11 specificities and will therefore react positively with some mixes (Table 3). Please take this into consideration while interpreting your reaction patterns!

- **Mix 16** may produce a relatively pronounced primer-dimer band. In order to clearly separate the allele-specific band (105 or 175 bp) from the primer-dimer band, one can e. g. extend the duration of the gel electrophoresis.

6. Trouble Shooting

Please refer to Section 8 of the 'Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No. 100A) supplied along with this product.

7. Precaution

Please refer to Material Safety Data Sheet for the CTS-PCR-SSP TRAY and MINITRAY KITS (Manual No. 100B) supplied along with this product.

8. Contact

If you have any particular questions concerning this kit, which are not answered in this or the Main Manual, please do not hesitate to contact me or my coworkers at:

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Hien Tran, M.D.

Table 1: Sizes of the PCR products and allele Specificities of each HLA-DRB1*11 SUBTYPING CTS-PCR-SSP primer mix (Lot No. DR11L04-4) based on the IMGT/HLA Sequence Database Release 2.24.0, January 2009
Amplification Control (Internal Positive Control): Product size 440 base pairs (bp)

Position on Tray			Primer Mix	Allele Specificity	Serology	Product sizes (bp)
H1	H5	H9	Mix 1 DR11L04-0	DRB1*0308, DRB1*110101-0103/0104/0105-0404/0601-21/23-29/31-35/36/37-48/49/50-64/66/68-70	-, DR11(5)	175
G1	G5	G9	Mix 2 DR11L04-0	DRB1*0831, DRB1*1105/29/47/63/67, DRB1*1204, DRB1*1411	-, DR11(5), DR14(6)	see below
				DRB1*0831, DRB1*1105/67, DRB1*1204, DRB1*1411	-, DR11(5), DR14(6)	175
				DRB1*1129/47/63	DR11(5), -	105
F1	F5	F9	Mix 3 DR11L04-3	DRB1*0415, DRB1*0831, DRB1*110101-0107/03-0602/09-1101/1201/1202/15/22-25/2701-30/32/33/35/37-41/43/44/46/47/49-51/5401/5402/56/58/60-62/66/67, DRB5*0113	DR4, -, DR11(5)	65
E1	E5	E9	Mix 4 DR11L04-4	DRB1*110201/0202/1401-16/18/1901/1902/20/21/31/45/48/6501/6502/70, DRB1*130101-0301/0302/04/06/08/10/12/13/15/16/19/22/23/27/28/30/31-39/40/41/45/48/51-53/57-59/61/62/64-66/68-70/72/73/76/79/80/82-84/87, DRB1*1416/63/67/78	DR11(5), -, DR13(6), DR6	see below
				DRB1*1115, DRB1*1362	-	115
				DRB1*110201/0202/1401/1402/16/1902/20/21/48/6501/6502/70, DRB1*130101-0301/0302/04/08/10/15/16/19/22/23/27/28/31-39/40/41/45/48/51-53/57/59/61/64-66/68-70/72/73/76/79/80/83/84/87, DRB1*1416	DR11(5), -, DR13(6), DR6	200
				DRB1*110201/0202/1401/1402/16/18/1901/1902/20/21/31/45/48, DRB1*130101-0202/0301/0302/04/06/08/10/12/13/15/16/19/22/23/27/28/30/31-39/40/41/45/48/51-53/57-59/61/64-66/68-70/82, DRB1*1416/63/67/78	DR11(5), -, DR13(6), DR6	200
D1	D5	D9	Mix 5 DR11L04-0	DRB1*0415/61, DRB1*0831, DRB1*110101/0102/0103/0104-0107/0401-0602/0801-1002/1201-1302/15/17-1901/1902/22-35/37-39/42-47/49/50/51/52/5401/55-58/60-62/64/66/67, DRB1*1204?, DRB1*1411, DRB5*0113?	DR4, -, DR11(5), DR14(6)	80/90
C1	C5	C9	Mix 6 DR11L04-0	DRB1*110201-03/1101/1102/1401/1402/16/20/21/36/40/41/48/59/63/6501/6502/68	DR11(5), -	80
B1	B5	B9	Mix 7 DR11L04-0	DRB1*0461, DRB1*110101-0107/05/0801-1202/1401/15/1901-20/22-24/26/2701/2702-33/37/39/45/49/51/53/61/62/64/66/68/69, DRB3*0218, DRB5*0113	-, DR11(5)	125
A1	A5	A9	Mix 8 DR11L04-0	DRB1*0308, DRB1*0415, DRB1*0831, DRB1*110201-0404/0601-07/1301/1302/16-18/21/25/34-36/38/40-44/46-48/50/52/5401-60/63/6501/6502/67/70, DRB1*1204, DRB1*1411	-, DR4, DR11(5), DR14(6)	125
H2	H6	H10	Mix 9 DR11L04-0	DRB1*0308, DRB1*1107/2701/2702, DRB3*0218	-, DR11(5)	85/95
G2	G6	G10	Mix 10 DR11L04-0	DRB1*1123/25/45/55/64/67	DR11(5), -	85
F2	F6	F10	Mix 11 DR11L04-0	DRB1*110601/0602/0801/0802/21/36/42/47/57/64, DRB1*1204	DR11(5), -	see below
				DRB1*110801/0802/36/42/57/64	DR11(5), -	65
				DRB1*110601/0602/21/47/57, DRB1*1204	DR11(5), -	120
E2	E6	E10	Mix 12 DR11L04-1	DRB1*0308, DRB1*110404/09-1002/1202-1302/16/17/20/22/40/46/52/58/59, DRB1*1411	-, DR11(5), DR14(6)	110

Position on Tray			Primer Mix	Allele Specificity	Serology	Product sizes (bp)
D2	D6	D10	Mix 13 DR11L04-1	DRB1*0308, DRB1*1109/16/20/28/30/40/59, DRB3*0218	-, DR11(5)	see below
				DRB1*1130, DRB3*0218	-	175
				DRB1*0308, DRB1*1109/16/20/28/40/59	-, DR11(5)	105
C2	C6	C10	Mix 14 DR11L04-0	DRB1*0424/69/78, DRB1*090102-0202/04-08, DRB1*100101/0102/02, DRB1*1117/52, DRB1*140101-0103/04-0503/0701-08/10/11/14/18/2301/2302/26/28/31-3202/34/36/38/39/43-45/50/54-56/58/59/61/62/68/70/71/75/82, DRB5*0112	DR4, -, DR9, DR10, DR14(6), DR1404, DR6	110
B2	B6	B10	Mix 15 DR11L04-1	DRB1*111001/1002/1201-1302/17/52, DRB1*1411	-, DR11(5), DR14(6)	105
A2	A6	A10	Mix 16 DR11L04-0	DRB1*0415/61, DRB1*1115/22/24	DR4, -	see below
				DRB1*0415/61, DRB1*1122	DR4, -	175
				DRB1*1115/24	-	105
H3	H7	H11	Mix 17 DR11L04-0	DRB1*0808/15?, DRB1*1131, DRB1*1345, DRB1*140701/0702/22/25/49/53, DRB3*0216	-, DR14(6)	120
G3	G7	G11	Mix 18 DR11L04-0	DRB1*1132/70	-	85
F3	F7	F11	Mix 19 DR11L04-0	DRB1*1133/35/6501/6502	-	180
E3	E7	E11	Mix 20 DR11L04-0	DRB1*0415/25/36/47/54, DRB1*080101-0203/0401-09/11/16/20-22/24/26/31, DRB1*0903, DRB1*1137/41/50/56, DRB1*130701/0702/26/47/49/55, DRB1*1415/22/25/27/73, DRB1*160101/0102/04/08, DRB5*010101-05/08N-10N/13	DR4, -, DR8, DR13(6), DR14(6), DR16(2), DR51, Null	100
D3	D7	D11	Mix 21 DR11L04-0	DRB1*1138	-	120
C3	C7	C11	Mix 22 DR11L04-0	DRB1*0317/37, DRB1*1139/43/50, DRB1*1459	-	155
B3	B7	B11	Mix 23 DR11L04-0	DRB1*1144/49/50	-	100
A3	A7	A11	Mix 24 DR11L04-0	DRB1*1151	-	220
H4	H8	H12	Mix 25 DR11L04-0	DRB1*1153, DRB3*0218?	-	85
G4	G8	G12	Mix 26 DR11L04-0	DRB1*0308, DRB1*1109-1002/1301/1302/16/17/20/40/46/52/62, DRB1*1204?, DRB1*1411, DRB3*0218?	-, DR11(5), DR14(6)	120
F4	F8	F12	Mix 27 DR11L04-0	DRB1*03010101-0106/04/07/08/10/11-13/15/16/18-25/28/30/32-34/36/37/39, DRB1*0442/59, DRB1*0828, DRB1*110201-0404/0601-07/1301/1302/16/18/21/25/34-36/38/40/42-44/46/47/5401-55/57-60/63/6501/6502/67/70, DRB1*120101-0201/0202/0203-10/12-15/17/18, DRB1*130101-0103/04/06/09/10-1102/15/17/18/20/22/24/27/28/35/42-44/51/52/54/57/59/61/64/68/69/71/75/77-80/83/87, DRB1*1417/21/33/35/64/65/72, DRB1*15010101-0106/030101-06/09/10/12/13/17N/18/20-25/28, DRB7*010101?	DR17(3), -, DR11(5), DR12(5), DR13(6), DR6, DR15(2), Null	160
E4	E8	E12	Mix 28 DR11L04-0	DRB1*110105/0403/0602/1301/1302/17/52/5401, DRB1*1204, DRB1*1411	DR11(5), -, DR14(6)	100
D4	D8	D12	Mix 29 DR11L04-0	DRB1*0310, DRB1*1152, DRB1*1343, DRB1*140101/0102/0103/04/08/10/16/26/28/31/3201/34/35/38/39/50/54/55	-, DR14(6), DR1404, DR6	120
C4	C8	C12	Mix 30 DR11L04-0	Negative Control	-	-

w = weak

? = nucleotide sequence information not available for the primer matching sequence

bold: specificities amplified by primer mixes which result in PCR fragments of different sizes

Table 2: Amplification patterns of HLA-DRB1*11 alleles detected by the HLA-DRB1*11 SUBTYPING CTS-PCR-SSP primer mixes (Lot No. DR11L04-4) Based on the IMGT/HLA Sequence Database Release 2.24.0, January 2009

Allele	Serology	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	29	
DRB1*110101/0102/0104/0106/0107/61/66	DR11(5), -	+		+		+		+																							
DRB1*110103	DR11(5)	+		+		w		+																							
DRB1*110105	DR11(5)	+		+				+																						+	
DRB1*110201/0202	DR11(5)	+			+			+		+																				+	
DRB1*1103	DR11(5)	+		+				+		+																				+	
DRB1*110401/0402/60	DR11(5), -	+		+		+				+																				+	
DRB1*110403/5401	DR11(5), -	+		+		+				+																				+	+
DRB1*110404/58	DR11(5), -	+		+		+				+			+																	+	
DRB1*1105	DR11(5)		+	+		+		+																							
DRB1*110601	DR11(5)	+		+		+				+		+																		+	
DRB1*110602	DR11(5)	+		+		+				+		+																		+	+
DRB1*1107	-	+								+	+																			+	
DRB1*110801/0802	DR11(5)	+				+		+				+																			
DRB1*1109	DR11(5)	+		+		+		+					+	+																+	
DRB1*111001/1002	-	+		+		+		+					+				+													+	
DRB1*111101	-	+		+				+	+																						
DRB1*111102/68	-	+						+	+																						
DRB1*111201	-	+		+		+		+									+														
DRB1*111202	-	+		+		+		+					+				+														
DRB1*111301/1302	DR11(5)	+				+				+			+				+												+	+	+
DRB1*111401	DR11(5)	+			+			+	+																						
DRB1*111402	DR11(5)	+			+			+																							
DRB1*1115	-	+		+	+	+		+									+														
DRB1*1116	-	+		+		+		+		+			+	+																+	+
DRB1*1117	-	+				+				+			+		+	+														+	+
DRB1*1118	-	+			+	+				+																				+	
DRB1*111901	-	+			+	+		+																							
DRB1*111902	-	+			+	w		+																							
DRB1*1120	DR11(5)	+			+			+	+				+	+																+	
DRB1*1121	DR11(5)	+			+			+		+		+																		+	
DRB1*1122	-			+		+		+					+					+													
DRB1*1123	DR11(5)	+		+		+		+			+																				
DRB1*1124	-	+		+		+		+									+														
DRB1*1125	DR11(5)	+		+		+		+		+		+																		+	
DRB1*1126	DR11(5)	+				+		+		+																					
DRB1*112701/2702	DR11(5)	+		+		+		+		+																					
DRB1*1128	-	+		+		+		+		+																					
DRB1*1129	DR11(5)	+	+	+		+		+		+																					
DRB1*1130	-			+		+		+		+					+																
DRB1*1131	-	+			+	+		+		+										+											
DRB1*1132	-	+		+		+		+		+											+										
DRB1*1133	-	+		+		+		+		+												+									
DRB1*1134	-	+				+				+																				+	
DRB1*1135	-	+		+		+		+		+												+								+	

Allele	Serology	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	29				
DRB1*1136	-	+					+		+			?																	+					
DRB1*1137	-	+		+		+		+														+												
DRB1*1138	-	+		+		+			+														+							+				
DRB1*1139	-	+		+		+		+																+										
DRB1*1140	-	+		+			+		+				+	+														+	+					
DRB1*1141	-	+		+			+		+													+												
<u>DRB1*1142/57</u>	-	+				+			+			+																		+				
DRB1*1143	-	+		+		+			+															+							+			
DRB1*1144	-	+		+		+			+																+						+			
DRB1*1145	-	+			+	+		+			+																							
DRB1*1146	-	+		+					+				+																+	+				
DRB1*1147	-	+	+	+		+			+			+																		+	+			
DRB1*1148	-	+			+		+		+																									
DRB1*1149	-	+		+		+		+																										
DRB1*1150	-	+		+		+			+													+		+	+									
DRB1*1151	-	+		+		w		+																	+									
DRB1*1152	-	+				+			+				+		+	+													+		+	+	+	
DRB1*1153	-	+						+																				+						
DRB1*115402	-	+		+					+																						+			
DRB1*1155	-	+				+			+		+																					+		
DRB1*1156	-	+		+		+			+													+												
DRB1*1159	-	+					+		+				+	+																		+		
DRB1*1162	-	+		+		+		+																						+				
DRB1*1163	-	+	+				+		+																							+		
DRB1*1164	-	+				+		+			+	+																						
DRB1*116501/6502	-				+		+		+													+										+		
DRB1*1167	-		+	+		+			+		+																					+		
DRB1*1169	-	+						+																										
DRB1*1170	-	+			+				+												+											+		

w = weak

? = nucleotide sequence information not available for the primer matching sequence

Underlined alleles = new HLA-DRB1*11 alleles that have identical amplification patterns as the previously known HLA-DRB1*11 alleles.

	Serology	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	29		
DRB1*1117	-	+				+			+				+		+	+											+		+			
DRB1*1118	-	+			+	+			+																				+			
DRB1*111901	-	+			+	+			+																				+			
DRB1*111902	-	+			+	w			+																							
DRB1*1120	DR11(5)	+			+		+	+					+	+													+					
DRB1*1121	DR11(5)	+			+		+	+				+																+				
DRB1*1122	-			+		+		+	+				+					+														
DRB1*1123	DR11(5)	+		+		+		+			+																					
DRB1*1124	-	+		+		+		+										+														
DRB1*1125	DR11(5)	+		+		+			+		+																		+			
DRB1*1126	DR11(5)	+				+		+																								
DRB1*112701/2702	DR11(5)	+	+		+	+	+	+	+	+																						
DRB1*1128	-	+		+		+		+	+					+																		
DRB1*1129	DR11(5)	+	+	+		+		+	+																							
DRB1*1130	-			+		+		+	+					+																		
DRB1*1131	-	+			+	+		+	+										+													
DRB1*1132	-	+		+		+		+	+											+												
DRB1*1133	-	+		+		+		+	+												+											
DRB1*1134	-	+				+			+																				+			
DRB1*1135	-	+		+		+			+												+								+			
DRB1*1136	-	+					+		+			?																	+			
DRB1*1137	DRB1*1137	+	+		+	+		+	+													+								+		
DRB1*1138	-	+		+		+			+														+							+		
DRB1*1139	-	+		+		+		+	+														+									
DRB1*1140	-	+		+			+		+				+	+														+	+			
DRB1*1141	-	+		+			+		+													+										
DRB1*1142/57	DRB1*1142/57	+	+			+		+	+			+																	+			
DRB1*1143	-	+		+		+			+														+							+		
DRB1*1144	-	+		+		+			+															+						+		
DRB1*1145	-	+			+	+		+			+																					
DRB1*1146	-	+		+		+		+	+				+															+	+			
DRB1*1147	DRB1*1147	+	+	+		+		+	+			+																		+		
DRB1*1148	-	+			+		+		+																							
DRB1*1149	-	+		+		+		+	+																							
DRB1*1150	-	+		+		+		+	+													+										
DRB1*1151	-	+		+		w		+	+																	+						
DRB1*1152	DRB1*1152	+	+			+		+	+			+		+	+	+												+		+	+	+
DRB1*1153	-	+						+																						+		
DRB1*115402	-	+		+					+																					+		
DRB1*1155	-	+				+			+		+																			+		
DRB1*1156	-	+		+		+			+													+										
DRB1*1159	DRB1*1159	+	+			+		+	+				+	+																+		
DRB1*1162	-	+		+		+		+	+																			+				
DRB1*1163	DRB1*1163	+	+					+	+																					+		
DRB1*1164	-	+				+		+	+		+	+																				
DRB1*116501/6502	DRB1*116501/6502	-			+		+	+	+												+									+		
DRB1*1167	DRB1*1167	-		+	+		+		+		+																			+		
DRB1*1169	DRB1*1169	-	+					+																								
DRB1*1170	DRB1*1170	-	+			+			+											+										+		
DRB1*1204	DRB1*1204	-		+		w			+		+																	w	+	+		
DRB1*130101-0103/04/06/10/15/22/27/28/35/51/52/57/59/61/64/68/69/79/80/83/87	DR13(6), -				+																								+			

Allele	Serology	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	29
DRB1*130201-0302/08/12/13/16/19/23/30-34/36-41/48/53/58/62/65/66/70/72/73/76/82/84, DRB1*1463/67/78	DR13(6), DR6, -				+																									
DRB1*1345	-				+													+												
DRB1*140101-0103/04/08/10/26/28/31/3201/34/38/39/50/54/55	DR14(6), DR1404, -														+															+
DRB1*140701/0702	DR14(6)														+			+												
DRB1*1411	DR14(6)		+			+			+				+		+	+											+		+	
DRB1*1416	DR6				+																									+
DRB1*1449/53, DRB3*0216	-																	+												
DRB1*1459	-														+								+							
DRB3*0218	-							+		+				+													w	w		
DRB5*0113	-			+		w		+													+									
DRB7*010101	-																												w	

w = weak

? = nucleotide sequence information not available for the primer matching sequence