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CTS *Collaborative Transplant Study*

WORKING INSTRUCTION

HLA-DRB1*15 SUBTYPING CTS-PCR-SSP MINITRAYS

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following products:

Product No.	Description	
215	HLA-DRB1*15 Subtyping CTS-PCR-SSP Minitray Kit	  0197

Changes compared to lot DR1516L04-1:

- In contrast to the previous lot, this kit contains only mixes for HLA-DRB1*15 subtyping only. For HLA-DRB1*16 subtyping, please use the product no. 216.
- Mix no. 3, 4, 5, 6, 7, 11, 15, 17 and 20 also amplify some HLA-DRB alleles other than HLA-DRB1*15. Please take this into consideration while interpreting your results.

Introduction

- Intended use: This kit reveals high resolution typing of HLA-DRB1*15 by the PCR-SSP method.
- Allele coverage: IMGT/HLA Sequence Database 2.24.0, January 2009
- This manual is only valid for Lot No. DR15L05-0.
- This manual should be used together with the Main Manual (General Information Sheet) titled '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 (minitray): 24 (23 allele-specific mixes and 1 negative control mix)
- Number of tests (minitrays) per kit: 12
- The primer mixes are aliquoted and lyophilized in thin-walled, green PCR-minitrays.
- PCR buffer: 1 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 the 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 also 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.

6. Troubleshooting

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*15 SUBTYPING CTS-PCR-SSP primer mix (Lot No. DR15L05-0) based on the IMGT/HLA Sequence Database 2.24.0, January 2009

Amplification Control (Internal Positive Control): Product size 440 base pairs (bp)

Primer mix	Allele specificity	Serology	Product sizes
Mix 1	DRB1*15010101-0106/030101-07/09/10/12/13/16-18/20-25, DRB4*0102?	DR15(2), -, Null, DR53	260
Mix 2	DRB1*150104/0202/0205/12	DR15(2), -	170/175
Mix 3	DRB1*150201/0202/0203/0204-0206/08/09/11/14/15/19/26/27/29-31, DRB1*160101/0102/0201/0202/03/04/0501/0502/07/08-11/13N, DRB4*0102?	DR15(2), DR2, -, DR16(2), Null, DR53	see below
	DRB1*1509, DRB4*0102?	-, DR53	145
	DRB1*150201/0202/0203/0204-0206/08/11/14/15/19/26/27/29-31, DRB1*160101/0102/0201/0202/03/04/0501/0502/07/08-11/13N	DR15(2), DR2, -, DR16(2), Null	260
Mix 4	DRB1*0106/09/15, DRB1*0473, DRB1*1424/37, DRB1*15030101/030102/07/11/23, DRB5*0106/11, DRB5*0202-04	-, DR15(2), DR51	see below
	DRB1*15030101/030102/23	DR15(2), -	165
	DRB1*0106/09/15, DRB1*0473, DRB1*1424/37, DRB1*1507/11, DRB5*0106/11, DRB5*0202-04	-, DR51	110
Mix 5	DRB1*1504/08/15, DRB1*1609/13N	DR15(2), DR2, -, Null	200
Mix 6	DRB1*0473, DRB1*1169, DRB1*1309, DRB1*1424/37, DRB1*15010101-0206/04-09/11-20/22/24/26/28-31	-, DR15(2), DR2, Null	165
Mix 7	DRB1*1505/23/25/27w/31, DRB1*160201/0202/10/11	DR15(2), -, DR16(2)	200
Mix 8	DRB1*1506	DR15(2)	150
Mix 9	DRB1*1512/13	-	170/195
Mix 10	DRB1*1514	-	240
Mix 11	DRB1*1504/15, DRB1*1609/13N	DR15(2), -, Null	200
Mix 12	DRB1*1516	-	120
Mix 13	DRB1*1517N	Null	210
Mix 14	DRB1*1518	-	210
Mix 15	DRB1*1309?, DRB1*1519	-	220
Mix 16	DRB1*1520	-	255
Mix 17	DRB1*1521, DRB1*1604	-, DR16(2)	225
Mix 18	DRB1*1522/30	-	165/175
Mix 19	DRB1*15030101/030102/23	DR15(2), -	165
Mix 20	DRB1*1426, DRB1*1524	DR14(6), -	225
Mix 21	DRB1*1530	-	175
Mix 22	DRB1*1526	-	235
Mix 23	DRB1*1529	-	175
Mix 24	Negative Control	-	-

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

w = weak

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

Table 2: Amplification patterns of HLA-DRB1*15 alleles detected by the HLA-DRB1*15 SUBTYPING CTS-PCR-SSP primer mixes (Lot No. DR15L05-0) based on the IMGT/HLA Sequence Database 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
DRB1*15010101-0103/0105/0106	DR15(2)	+					+																	
DRB1*150104	DR15(2)	+	+				+																	
DRB1*150201/0203/0204/0206	DR15(2)			+			+																	
DRB1*150202/0205	DR15(2)		+	+			+																	
DRB1*15030101/030102	DR15(2)	+			+																+			
DRB1*1504	DR15(2)	+				+	+					+												
DRB1*1505	DR15(2)	+					+	+																
DRB1*1506	DR15(2)	+					+		+															
DRB1*1507	-	+			+		+																	
DRB1*1508	DR2			+		+	+																	
DRB1*1509	-	+		+			+																	
DRB1*1510	-	+																						
DRB1*1511	-			+	+		+																	
DRB1*1512	-	+	+				+			+														
DRB1*1513	-	+					+			+														
DRB1*1514	-			+			+				+													
DRB1*1515	-			+		+	+				+													
DRB1*1516	-	+					+						+											
DRB1*1517N		+					+							+										
DRB1*1518	-	+					+								+									
DRB1*1519	-			+			+									+								
DRB1*1520	-	+					+										+							
DRB1*1521	-	+																+						
DRB1*1522	-	+					+												+					
DRB1*1523	-	+			+			+													+			
DRB1*1524	-	+					+															+		
DRB1*1525	-	+						+																
DRB1*1526	-			+			+																	+
DRB1*1527	-			+				w																
DRB1*1528	-						+																	
DRB1*1529	-			+			+																	+
DRB1*1530	-			+			+												+			+		
DRB1*1531	-			+			+	+																

w = weak

Table 3: Amplification patterns of HLA-DRB1*15 and other HLA-DRB alleles detected by the HLA-DRB1*15 SUBTYPING CTS-PCR-SSP primer mixes (Lot No. DR15L05-0) based on the IMGT/HLA Sequence Database 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
DRB1*0106/09/15, DRB5*0106/11, DRB5*0202-04	-, DR51				+																			
DRB1*0473, DRB1*1424/37	-				+		+																	
DRB1*1309	-						+									?								
DRB1*1426	DR14(6)																					+		
DRB1*15010101-0103/0105/0106	DR15(2)	+					+																	
DRB1*150104	DR15(2)	+	+				+																	
DRB1*150201/0203/0204/0206	DR15(2)			+			+																	
DRB1*150202/0205	DR15(2)		+	+			+																	
DRB1*15030101/030102	DR15(2)	+			+																+			
DRB1*1504	DR15(2)	+				+	+					+												
DRB1*1505	DR15(2)	+					+		+															
DRB1*1506	DR15(2)	+					+			+														
DRB1*1507	-	+			+		+																	
DRB1*1508	DR2			+		+	+																	
DRB1*1509	-	+		+			+																	
DRB1*1510	-	+																						
DRB1*1511	-			+	+		+																	
DRB1*1512	-	+	+				+			+														
DRB1*1513	-	+					+			+														
DRB1*1514	-			+			+				+													
DRB1*1515	-			+		+	+					+												
DRB1*1516	-	+					+						+											
DRB1*1517N		+					+							+										
DRB1*1518	-	+					+								+									
DRB1*1519	-			+			+									+								
DRB1*1520	-	+					+										+							
DRB1*1521	-	+																	+					
DRB1*1522	-	+					+													+				
DRB1*1523	-	+			+			+													+			
DRB1*1524	-	+					+															+		
DRB1*1525	-	+						+																
DRB1*1526	-			+			+																+	
DRB1*1527	-			+				w																
DRB1*1169, DRB1*1528	-						+																	
DRB1*1529	-			+			+																	+
DRB1*1530	-			+			+												+			+		
DRB1*1531	-			+			+	+																
DRB1*160101/0102/03/0501/0502/07/08	DR16(2), DR2, -			+																				
DRB1*160201/0202/10/11	DR16(2)			+				+																
DRB1*1604	DR16(2)			+															+					
DRB1*1609/13N	-			+		+						+												
DRB4*0102	DR53	?		?																				

w = weak

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