

Olerup SSP[®] DRB5

Product number:	101.123-24/06 – including <i>Taq</i> pol.
Lot number:	36F
Expiry date:	2010-November-01
Number of tests:	24 test – Product No. 101.123-24 6 tests – Product No. 101.123-06
Number of wells per test:	16
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

This Product Description is only valid for Lot No. 36F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP[®] DRB5* LOT

The DRB5 specificity and interpretation tables have been updated for the HLA-DRB alleles described since the previous *Olerup SSP[®] DRB5* lot was made (**Lot No. 28E**).

The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot.

Well	5'-primer	3'-primer	rationale
6	Modified	-	Modified 5'-primer to decrease primer dimer formation.

PRODUCT DESCRIPTION

DRB5 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB5*0101 to DRB5*0113 and the DRB5*0202 to DRB5*0205 alleles.

PLATE LAYOUT

Each test consists of 16 PCR reactions in a 16 well cut PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16

The 16 well cut PCR plate is marked with 'DRB5' in silver/gray ink.

Well No. 1 is marked with the Lot No. '36F'.

The PCR plates are covered with a PCR-compatible foil.

Please note: When removing each 16 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Only alleles of the DRB5 locus will be amplified by the DRB5 subtyping kit, except that the DRB1*0907 allele will be amplified by primer mixes 1, 2, 3 and 15.

UNIQUELY IDENTIFIED ALLELES

All the DRB5 alleles, i.e. **DRB5*0101 to DRB5*0113 and DRB5*0202 to DRB5*0205**, recognized by the HLA Nomenclature Committee in October 2008¹ will give rise to unique amplification patterns by the primers in the DRB5 subtyping kit².

¹DRB5 alleles listed on the IMGT/HLA web page 2008-October-10, release 2.23.0, www.ebi.ac.uk/imgt/hla.

²The DRB5*0112 and DRB1*0907 alleles will give rise to identical amplification patterns. These two alleles can be distinguished by e.g. the DR low resolution primer set.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 17 phenotypically different DRB5 alleles generate 18 amplification patterns, that can be combined in 171 homozygous and heterozygous combinations. Sixty-three of these genotypes do not give rise to unique amplification patterns.

++++++-+	--+-----	0105,0106 = 0105,0111
+++++--+	+--+-----	0106,0113 = 0111,0113
+++++--+	-++-----	0106,0107 = 0107,0111 = 0107,0203
+++++--+	--+---+-	0106,0109 = 0109,0111 = 0109,0203
+++++--+	--+-----	010101,0106 = 010101,0111 = 010101,0203
+++++---	++-----	0104,0107 = 0107,0113
+++++---	+-----+-	0104,0109 = 0109,0113
+++++---	+-----	010101,0104 = 0101,0113 = 0104,0113
+++++---	-+-----	0101,0107 = 0107,0107
+++++---	-----+-	0101,0109 = 0109,0109
++++-+++	--+---+-	0106,0108N = 0108N,0111
++++-+++	--+---+-	0106,0110N = 0110N,0111
++++-+++	--+-----	0102,0106 = 0102,0111
++++-+++	+--+-----	0104,0106 = 0104,0111 = 0104,0203
++++-+++	--+---+-	0106,0112 = 0111,0112
++++-+++	--+-----	010102,0106 = 010102,0111 = 010102,0203
++++----	+-----	010102,0104 = 0104,0104
+++--+++	-----+-	0103,0110N = 0103,0112
+++--++-	-----+-	0108N,0110N = 0108N,0112
+++--++-	-----+-	0102,0108N = 0108N,0108N
+++--++-	-----+-	0102,0110N = 0102,0112 = 0110N,0110N = 0110N,0112
+++--+++	--+-----	0103,0106 = 0103,0111
+--+-----	--+-----	0106,0111 = 0106,0203 = 0111,0111 = 0111,0203

SPECIFICITY TABLE

DRB5 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for DRB5 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB5 ³ alleles	Other amplified DRB alleles ⁴
1	255 bp	515 bp	010101-0105, 0107-0113, 0203	DRB1*0907
2⁵	210 bp	515 bp	010101-0105, 0107-0110N, 0112, 0113, 0204	DRB1*0907
3⁷	225 bp	430 bp	010101-0102, 0104, 0105, 0107-0110N, 0112, 0113, 0205	DRB1*0907
4⁶	100 bp	430 bp	010101-010102, 0104, 0106, 0107, 0109, 0111	
5	150 bp	515 bp	010101, 0105, 0107, 0109, 0113	
6⁶	145 bp	430 bp	0102, 0103, 0105, 0108N, 0110N	
7	150 bp	430 bp	0102, 0103, 0108N, 0110N	
8⁸	215 bp	430 bp	0103, 0106, 0111, 0202-0204	
9⁹	175, 225 bp	430 bp	0104, 0113	
10⁶	130 bp	430 bp	0107	
11	200 bp	430 bp	0106, 0111, 0202, 0203	
12	185 bp	515 bp	0202, 0204, 0205	
13	195 bp	430 bp	0108N	
14	210 bp	430 bp	0109	
15	240 bp	430 bp	0110N, 0112	DRB1*0907
16⁶	140 bp	430 bp	0205	

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Lot-specific information

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¹Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of DRB5 SSP subtypings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective length of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB5 subtyping.

In addition, wells number 2, 5 and 12 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

In the presence of a specific amplification the intensity of the control band often decreases.

³For several DRB alleles only partial second exon nucleotide sequences are available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. We assume that unknown sequences in the first hyperpolymorphic region of the second exon of DRB alleles are conserved within allelic groups and that unknown sequences of codons 87 to 92 are identical with the DRB1*0101 consensus sequence.

⁴Due to the sharing of sequence motifs between DRB alleles non-DRB5 alleles will be amplified by primer mixes 1 to 3 and 15.

The DRB5*0112 and DRB1*0907 alleles will give rise to identical amplification patterns. These two alleles can be distinguished by e.g. the DR low resolution primer set.

⁵Primer mix 2 may give rise to less specific PCR product than the other DRB5 primer mixes.

⁶Specific PCR fragments shorter than 150 base pairs are less intense and not as sharp as longer specific bands.

⁷Primer mix 3 frequently gives rise to an extra band longer than the control band. This band should be disregarded in the interpretation of DRB5 SSP typings.

⁸Primer mix 8 may give rise to primer dimer formation.

⁹Primer mix 9. Specific PCR fragment of 175 bp in the DRB5*0113 allele. Specific PCR fragment of 225 bp in the DRB5*0104 allele.

INTERPRETATION TABLE								
DRB5 SSP subtyping								
Amplification patterns of DRB5*0101 to 0205 alleles								
	Well⁶							
	1	2	3	4	5	6	7	8
Length of spec.	255	210	225	100	150	145	150	215
PCR product								
Length of int.	515	515	430	430	515	430	430	430
pos. control¹								
5'-primer(s)²	13	13	13	38	37	38	37	13
	5'-g TA 3'	5'-g TA 3'	5'-g TA 3'	5'-AC T 3'	5'-Ag g 3'	5'-AC g 3'	5'-Ag A 3'	5'-g TA 3'
	13	13				38		13
	5'-g TA 3'	5'-g TA 3'				5'-AC g 3'		5'-g TA 3'
3'-primer(s)³	85	67	71	57	72	72	72	71
	5'-C AA 3'	5'-gAA 3'	5'-g CC 3'	5'-gC g 3'	5'-gC g 3'	5'-gC g 3'	5'-gC g 3'	5'-g Cg 3'
		72	74					71
		5'-gC g 3'	5'-CAg 3'					5'-g Cg 3'
			78					
			5'-CAC 3'					
Well No.	1	2	3	4	5	6	7	8
DRB5 allele⁴								
*010101	1	2	3	4	5			
*010102	1	2	3	4				
*0102	1	2	3			6	7	
*0103	1	2				6	7	8
*0104	1	2	3	4				
*0105	1	2	3		5	6		
*0106				4				8
*0107	1	2	3	4	5			
*0108N	1	2	3			6	7	
*0109	1	2	3	4	5			
*0110N	1	2	3			6	7	
*0111	1			4				8
*0112, DRB1*0907⁵	1	2	3					
*0113	1	2	3		5			
*0202								8
*0203	1							8
*0204		2						8
*0205			3					
DRB5 allele⁴								
Well No.	1	2	3	4	5	6	7	8

INTERPRETATION TABLE								
DRB5 SSP subtyping								
Amplification patterns of DRB5*0101 to 0205 alleles								
Well ⁶								
9	10	11	12	13	14	15	16	
175	130	200	185	195	210	240	140	Length of spec.
225								PCR product
430	430	430	515	430	430	430	430	Length of int.
								pos. control ¹
13	38	13	37	108	13	13	37	5'-primer(s) ²
5'-g TA ^{3'}	5'-AC T ^{3'}	5'-g TA ^{3'}	5'-Ag A ^{3'}	5'-Ag A ^{3'}	5'-g TA ^{3'}	5'-g TA ^{3'}	5'-Ag A ^{3'}	
58	67	67	85	160	70	78	70	3'-primer(s) ³
5'-C CT ^{3'}	5'-gAT ^{3'}	5'-gAT ^{3'}	5'-C Ag ^{3'}	5'-CAT ^{3'}	5'-gTT ^{3'}	5'-CAC ^{3'}	5'-CTg ^{3'}	
74						79		
5'-CAG ^{3'}						5'-T gC ^{3'}		
9	10	11	12	13	14	15	16	Well No.
								DRB5 allele ⁴
								*010101
								*010102
								*0102
								*0103
9								*0104
								*0105
		11						*0106
10								*0107
				13				*0108N
				14				*0109
						15		*0110N
		11						*0111
						15		*0112, DRB1*0907 ⁵
9								*0113
		11	12					*0202
		11						*0203
			12					*0204
			12				16	*0205
								DRB5 allele ⁴
9	10	11	12	13	14	15	16	Well No.

Lot No.: 36F

Lot-specific information

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¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB5 subtyping.

In addition, wells number 2, 5 and 12 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

²The codon, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.

³The codon, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.

⁴The DRB5*0201 allele has been shown to be identical to DRB5*0202.

⁵The DRB5*0112 and DRB1*0907 alleles will give rise to identical amplification patterns. These two alleles can be distinguished by e.g. the DR low resolution primer set.

⁶Primer mix 9. Specific PCR fragment of 175 bp in the DRB5*0113 allele. Specific PCR fragment of 225 bp in the DRB5*0104 allele.

CELL LINE VALIDATION SHEET																
DRB5 SSP subtyping kit																
				Well												
					1	2	3	4	5	6	7	8	9	10	11	12
			Prod. No.:		200843301	200843302	200843303	200843304	200843305	200853906	200843307	200843308	200843309	200843310	200843311	200843312
					200843313	200843314	200843315	200843316								
	IHWC cell line	DRB5														
1	9001 SA			-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707	*0102		+	+	+	-	-	+	+	-	-	-	-	-	-
3	9011 E4181324	*0102		+	+	+	-	-	+	+	-	-	-	-	-	-
4	9275 GU373			-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*0202		-	-	-	-	-	-	-	+	-	-	+	+	-
6	9353 SM			-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL			-	-	-	-	-	-	-	-	-	-	-	-	-
8	9007 DEM	*0202		-	-	-	-	-	-	-	+	-	-	+	+	-
9	9026 YAR			-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3			-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT			-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB			-	-	-	-	-	-	-	-	-	-	-	-	-
13	9067 BTB			-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA			-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB			-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007			-	-	-	-	-	-	-	-	-	-	-	-	-
17	9008 WILJON	*0101		+	+	+	+	+	-	-	-	-	-	-	-	-
18	9257 32367			-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16			-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005			-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA			-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE			-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL			-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH			-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9			-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49			-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007			-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB			-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU			-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH			-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF			-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG			-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B			-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF			-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO			-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17			-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB			-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL			-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML			-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199			-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301			-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089			-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526			-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM			-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO			-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU	*0101		+	+	+	+	+	-	-	-	-	-	-	-	-
47	9045 TUBO			-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND			-	-	-	-	-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® DRB5 SSP

Product number: 101.123-24/06 – including *Taq* pol.
Lot number: 36F
Expiry date: 2010-November-01
Number of tests: 24 test – Product No. 101.123-24
6 tests – Product No. 101.123-06
Number of wells per test: 16

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2008-433-01	9	2008-433-09
2	2008-433-02	10	2008-433-10
3	2008-433-03	11	2008-433-11
4	2008-433-04	12	2008-433-12
5	2008-433-05	13	2008-433-13
6	2008-539-06	14	2008-433-14
7	2008-433-07	15	2008-433-15
8	2008-433-08	16	2008-433-16

The specificity of each primer solution of the kit has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 9, 10, 14, 15 and 16 were available. The specificities of the primers in primer solutions 9, 10 and 16 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 14 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solution 15 it was only possible to test the 5'-primer and one of the 3'-primers, one 3'-primer was not possible to test. Additional 3'-primers in primer solutions 2 and 3 were tested by separately adding additional 5'-primers.

Results: No false positive or false negative amplifications were obtained.

Date of approval: 2009-May-25

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DRB5
Product number: 101.123-24/06
Lot number: 36F

Intended use: DRB5 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Hasselstigen 1
SE-133 33 Saltsjöbaden, Sweden
Phone: +46-8-717 88 27
Fax: +46-8-717 88 18

We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2000 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, conformity assessed using Annex IV, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom. (Notified Body number: 0088.)

Saltsjöbaden, Sweden
2009-May-25

Olle Olerup
Managing Director

Lot No.: **36F**

Lot-specific information

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