

Lot No.: **50F**

Lot-specific information

Olerup SSP[®] DRB1*01

Product number: 101.111-24u/06u – without *Taq* pol.
Lot number: 50F
Expiry date: 2011-January-01
Number of tests: 24 test – Product No. 101.111-24u
6 tests – Product No. 101.111-06u
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. 50F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] DRB1*01 Lot

The DRB1*01 specificity and interpretation tables have been updated for the DRB1 alleles described since the previous *Olerup SSP*[®] DRB1*01 lot was made (**Lot No. 19E**).

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

Well	5'-primer	3'-primer	rationale
9	-	Added	Primer added for the DRB1*0120 allele.

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PRODUCT DESCRIPTION

DRB1*01 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*0101 to DRB1*0120 alleles.

STRIP LAYOUT

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

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

The 16 well PCR plate is marked with 'DRB1*01' in silver/gray ink.

Well No. 1 is marked with the Lot No. '50F'.

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 the DRB1*01 alleles will be amplified by the DRB1*01 subtyping kit. Thus, the interpretation of DRB1*01 subtypings is not influenced by other groups of DRB1 alleles.

UNIQUELY IDENTIFIED ALLELES

All the DRB1*01 alleles, i.e. **DRB1*0101 to DRB1*0120**, recognized by the HLA Nomenclature Committee in January 2009¹ will give rise to unique amplification patterns by the primers in the DRB1*01 subtyping kit.

The DRB1*01 subtyping kit cannot distinguish the DRB1*010101 to DRB1*010109 alleles or the DRB1*010201 to DRB1*010205 alleles.

¹DRB1 alleles listed on the IMGT/HLA web page 2009-January-16, release 2.24.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 20 DRB1*01 alleles can be combined in 210 homozygous and heterozygous combinations. Eighty of these genotypes do not give rise to unique amplification patterns. The different sizes of the specific PCR products generated by primer mixes 9 and 11 have not been considered in this calculation.

+ - + - - + -	+ - - - - - -	0103, 0108 = 0103, 0120
+ - + - - + -	- - + - - - -	0103, 0110 = 0103, 0118 = 0110, 0117
+ - + - - + -	- - - - - - -	0101, 0103 = 0103, 0117
+ - + - - - -	+ - + - - - -	0108, 0110 = 0110, 0120
+ - + - - - -	- - + - - - -	0101, 0110 = 0110, 0110 = 0110, 0118
+ - + - + - -	+ + - - - - -	0104, 0109 = 0106, 0111

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+--+--	+-----	0101,0104 = 0104,0108 = 0104,0111 =
		0108,0111 = 0111,0120
+--+--	-----	0101,0111 = 0111,0111
+--+--	+-----	0105,0108 = 0105,0120
+--+--	-----	0101,0105 = 0105,0105
+--+--	++-----	0106,0115 = 0106,0117 = 0108,0115 =
		0115,0120
+--+--	+-----	0108,0117 = 0117,0120
+--+--	-+-----	0101,0115 = 0109,0115 = 0109,0117 =
		0115,0117
+--+--	-----	0101,0117 = 0117,0117
+--+--	+-----	0107,0108 = 0107,0120
+--+--	-----	0101,0107 = 0107,0107
+--+--	++-----	0101,0106 = 0106,0108 = 0106,0109 =
		0108,0109 = 0109,0120
+--+--	+--+-----	0108,0118 = 0118,0120
+--+--	+--+-----	0108,0112 = 0112,0120
+--+--	+--+-----	0108,0113 = 0113,0120
+--+--	+--+-----	0108,0114 = 0114,0120
+--+--	+--+-----	0108,0116 = 0116,0120
+--+--	+--+-----	0108,0119 = 0119,0120
+--+--	+-----	0101,0108 = 0101,0120 = 0108,0108 =
		0108,0120
+--+--	-+-----	0101,0109 = 0109,0109
+--+--	--+-----	0101,0118 = 0118,0118
+--+--	---+-----	0101,0112 = 0112,0112
+--+--	----+-----	0101,0113 = 0113,0113
+--+--	-----+--	0101,0114 = 0114,0114
+--+--	-----+--	0101,0116 = 0116,0116
+--+--	-----+--	0101,0119 = 0119,0119
--+--	+-----	0104,0104 = 0104,0120
--+--	++-----	0106,0106 = 0106,0120

0101 = 010101-010109
0102 = 010201-010205

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SPECIFICITY TABLE

DRB1*01 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for DRB1*01 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB1*01 ³ alleles
1⁴	255 bp	515 bp	010101-010109, 0103, 0105, 0107-0119
2	235 bp	430 bp	010201-010205
3	200 bp	515 bp	010101-010205, 0104-0114, 0116-0120
4	210 bp	430 bp	0103, 0110
5	230 bp	430 bp	0104, 0111
6	130 bp	430 bp	0105
7	210 bp	430 bp	0103, 0115, 0117
8	210 bp	430 bp	0107
9^{5,6}	110, 255 bp	430 bp	0104, 0106, 0108, 0120
10	210 bp	430 bp	0106, 0109, 0115
11^{4,7}	140, 210 bp	430 bp	0110, 0118
12	215 bp	430 bp	0112
13⁵	85 bp	430 bp	0113
14	170 bp	430 bp	0114
15	215 bp	430 bp	0116
16	225 bp	430 bp	0119

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 DRB1*01 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 DRB1*01 subtyping.

In addition, well number 3 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

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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.

⁴Primer mixes 1 and 11 have a tendency of giving rise to primer oligomer formation.

⁵Specific PCR fragments shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR bands.

⁶Primer mix 9: Specific PCR fragment of 110 bp in the DRB1*0108 allele. Specific PCR fragment of 255 bp in the DRB1*0104, 0106 and 0120 alleles.

⁷Primer mix 11: Specific PCR fragment of 140 bp in the DRB1*0118 allele. Specific PCR fragment of 210 bp in the DRB1*0110 allele.

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INTERPRETATION TABLE								
DRB1*01 SSP subtyping								
Amplification patterns of the DRB1*0101 to 0120 alleles								
	Well⁴							
	1	2	3	4	5	6	7	8
Length of spec.	255	235	200	210	230	130	210	210
PCR product								
Length of int.	515	430	515	430	430	430	430	430
pos. control¹								
5'-primer²	14	14	14	14	14	14	14	10
	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gg g ^{3'}
3'-primer(s)³	86	78	67	71	77	45	67	67
	5'-C AC ^{3'}	5'-CA A ^{3'}	5'-gAg ^{3'}	5'-gCT ^{3'}	5'-A AT ^{3'}	5'-CCT ^{3'}	5'-gAT ^{3'}	5'-gAg ^{3'}
							74	
							5'-C CT ^{3'}	
Well No.	1	2	3	4	5	6	7	8
DRB1 allele								
*010101-010109	1		3					
*010201-010205		2	3					
*0103	1			4			7	
*0104			3		5			
*0105	1		3			6		
*0106			3					
*0107	1		3					8
*0108	1		3					
*0109	1		3					
*0110	1		3	4				
*0111	1		3		5			
*0112	1		3					
*0113	1		3					
*0114	1		3					
*0115	1						7	
*0116	1		3					
*0117	1		3				7	
*0118	1		3					
*0119	1		3					
*0120			3					
DRB1 allele								
Well No.	1	2	3	4	5	6	7	8

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INTERPRETATION TABLE								
DRB1*01 SSP subtyping								
Amplification patterns of the DRB1*0101 to 0120 alleles								
Well⁴								
9	10	11	12	13	14	15	16	
110	210	140	215	85	170	215	225	Length of spec.
255		210						PCR product
430	430	430	430	430	430	430	430	Length of int.
								pos. control¹
14	14	14	14	14	14	14	14	5'-primer²
5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	
37	71	47	72	30	57	73	76	3'-primer(s)³
5'-C gT ^{3'}	5'-CgC ^{3'}	5'-g gA ^{3'}	5'-C CA ^{3'}	5'-gAg ^{3'}	5'-C Ag ^{3'}	5'-g gC ^{3'}	5'-gTT ^{3'}	
85		71						
5'-CA A ^{3'}		5'-CTT ^{3'}						
9	10	11	12	13	14	15	16	Well No.
								DRB1 allele
								*010101-010109
								*010201-010205
								*0103
9								*0104
								*0105
9	10							*0106
								*0107
9								*0108
	10							*0109
		11						*0110
								*0111
			12					*0112
				13				*0113
					14			*0114
	10							*0115
						15		*0116
								*0117
		11						*0118
							16	*0119
9								*0120
								DRB1 allele
9	10	11	12	13	14	15	16	Well No.

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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 DRB1*01 subtyping.

In addition, well number 3 contains 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 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 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.

⁴Primer mix 9: Specific PCR fragment of 110 bp in the DRB1*0108 allele. Specific PCR fragment of 255 bp in the DRB1*0104, 0106 and 0120 alleles.

Primer mix 11: Specific PCR fragment of 140 bp in the DRB1*0118 allele. Specific PCR fragment of 210 bp in the DRB1*0110 allele.

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CELL LINE VALIDATION SHEET																	
DRB1*01 SSP subtyping kit																	
					Production No.	Well											
						1	2	3	4	5	6	7	8	9	10	11	12
						200842401	200842402	200842403	200842404	200842405	200842406	200842407	200842408	200855509	200842410	200842411	200842412
						200842413	200842414	200842415	200842416								
	IHWC cell line	DRB1	DRB1														
1	9001 SA	*0101				+	-	+	-	-	-	-	-	-	-	-	-
2	9280 LK707	*1502	*0405			-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324	*1502				-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373	*0301				-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*1601				-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM	*0407	*0803			-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL	*0301				-	-	-	-	-	-	-	-	-	-	-	-
8	9007 DEM	*0401	*1602			-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*0402				-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3	*0405				-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT	*0701				-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*0701				-	-	-	-	-	-	-	-	-	-	-	-
13	9067 BTB	*0801				-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*0802				-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*0901				-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*1101				-	-	-	-	-	-	-	-	-	-	-	-
17	9008 WILJON	*1501				-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*0901	*1101			-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16	*1201				-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*1302				-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*1402				-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*1302	*1401			-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*0803	*1414			-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*1101				-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*0701				-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*0301				-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*0405	*1001			-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB	*0416	*0701			-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*0701				-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*0302				-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*0301				-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*1303				-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*0404				-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*1101				-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*0403				-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*0403	*0406			-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB	*1301				-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*1402				-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML	*0301	*0401			-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*0701	*0901			-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*1302				-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*0803				-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*0901				-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*1401				-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*0701				-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU	*1501				-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*1104	*1201			-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND	*0103				+	-	-	+	-	-	+	-	-	-	-	-

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CERTIFICATE OF ANALYSIS

Olerup SSP® DRB1*01 SSP

Product number: 101.111-24u/06u – without *Taq* pol.
Lot number: 50F
Expiry date: 2011-January-01
Number of tests: 24 test – Product No. 101.111-24u
6 tests – Product No. 101.111-06u
Number of wells per test: 16

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2008-424-01	9	2008-555-09
2	2008-424-02	10	2008-424-10
3	2008-424-03	11	2008-424-11
4	2008-424-04	12	2008-424-12
5	2008-424-05	13	2008-424-13
6	2008-424-06	14	2008-424-14
7	2008-424-07	15	2008-424-15
8	2008-424-08	16	2008-424-16

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 5, 6, 8 to 16 were available. The specificities of the primers in primer solutions 5, 9 to 11 and 13 to 15 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 6, 12 and 16 it was only possible to test the 5'-primers, the 3'-primers was not possible to test. In primer solution 8 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. Additional 3'-primers in primer solution 7 were tested by separately adding one additional 5'-primer.

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

Date of approval: 2009-May-26

Approved by:

Quality Control, Supervisor

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Lot-specific information

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*01
Product number: 101.111-24u/06u
Lot number: 50F

Intended use: DRB1*01 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-26

Olle Olerup
Managing Director

Lot No.: **50F**

Lot-specific information

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For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.