

Olerup SSP® DRB5

Product number:	101.123-24u/06u – without <i>Taq</i> pol.
Lot number:	88G
Expiry date:	2012-February-01
Number of tests:	24 test – Product No. 101.123-24u
	6 tests – Product No. 101.123-06u
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. 88G.

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. 36F**).

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

Well	5'-primer	3'-primer	rationale
10	-	Added	Primer added for the DRB5*0112 allele.
14	-	Added	Primer added for the DRB5*0114 allele.

PRODUCT DESCRIPTION

DRB5 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB5*0101 to DRB5*0114 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. '88G'.

A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.

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*0114 and DRB5*0202 to DRB5*0205**, recognized by the HLA Nomenclature Committee in January 2010¹ will give rise to unique amplification patterns by the primers in the DRB5 subtyping kit.

¹DRB5 alleles listed on the IMGT/HLA web page 2010-January-15, release 2.28.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 18 phenotypically different DRB5 alleles generate 19 amplification patterns, that can be combined in 190 homozygous and heterozygous combinations. Sixty-seven of these genotypes do not give rise to unique amplification patterns. In these calculations the different sizes of the specific PCR products in lanes 9, 10 and 14 were not considered.

++++++--	0105,0106 = 0105,0111
++++++--	010101,0105 = 010102,0105
++++++--	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 = 010101,0113 = 010102,0113 =
	0104,0113
++++++--	0107,0109 = 0107,0114
++++++--	010101,0112 = 0107,0112
++++++--	010101,0107 = 010102,0107 = 0107,0107
++++++--	010101,0109 = 010101,0114 = 010102,0109 =
	0109,0109 = 0109,0114
++++++--	010101,010101 = 010101,010102
++++++--	0106,0108N = 0108N,0111
++++++--	0106,0110N = 0110N,0111
++++++--	0102,0106 = 0102,0111
++++++--	0104,0106 = 0104,0111 = 0104,0203
++++++--	0106,0112 = 0111,0112
++++++--	0106,0114 = 0111,0114
++++++--	010102,0106 = 010102,0111 = 010102,0203
++++++--	010102,0104 = 0104,0104
++++++--	0102,0112 = 0110N,0112
++++++--	0102,0108N = 0108N,0108N
++++++--	0102,0110N = 0110N,0110N
++++++--	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, 0107-0114, 0203	DRB1*0907
2⁵	210 bp	515 bp	*010101-0105, 0107-0110N, 0112-0114, 0204	DRB1*0907
3⁷	225 bp	430 bp	*010101-0102, 0104, 0105, 0107-0110N, 0112-0114, 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	
10^{6,10}	130, 160 bp	430 bp	*0107, 0112	
11	200 bp	430 bp	*0106, 0111, 0202, 0203	
12	185 bp	515 bp	*0202, 0204, 0205	
13	195 bp	430 bp	*0108N	
14^{6,11}	110, 210 bp	430 bp	*0109, 0114	
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.

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.

¹⁰Primer mix 10: Specific PCR fragment of 130 bp in the DRB5*0107 allele. Specific PCR fragment of 160 bp in the DRB5*0112 allele.

¹¹Primer mix 14: Specific PCR fragment of 110 bp in the DRB5*0114 allele. Specific PCR fragment of 210 bp in the DRB5*0109 allele.

INTERPRETATION TABLE								
DRB5 SSP subtyping								
Amplification patterns of DRB5*0101 to 0205 alleles								
	Well⁵							
	1	2	3	4	5	6	7	8
Length of spec.	255	210	225	100	150	145	150	215
PCR product(s)								
Length of int.	515	515	430	430	515	430	430	430
pos. control¹								
5'-primer(s)²	13(125)	13(125)	13(125)	38(199)	37(196)	38(199)	37(196)	13(125)
	5' -gTA 3'	5' -gTA 3'	5' -gTA 3'	5' -ACT 3'	5' -Agg 3'	5' -ACg 3'	5' -AgA 3'	5' -gTA 3'
						38(199)		
						5' -ACg 3'		
3'-primer(s)³	85(341)	67(286)	71(299)	57(258)	72(303)	72(303)	72(303)	71(299)
	5' -CAA 3'	5' -gAA 3'	5' -gCC 3'	5' -gCg 3'	5' -gCg 3'	5' -gCg 3'	5' -gCg 3'	5' -gCg 3'
		67(286)	74(307)					71(299)
		5' -gAA 3'	5' -CAg 3'					5' -gCg 3'
		70(296)	78(319)					
		5' -TCC 3'	5' -CAC 3'					
		72(303)						
		5' -gCg 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	1	2	3					
*0113	1	2	3		5			
*0114	1	2	3					
*0202								8
*0203	1							8
Well No.	1	2	3	4	5	6	7	8

INTERPRETATION TABLE								
DRB5 SSP subtyping								
Amplification patterns of DRB5*0101 to 0205 alleles								
Well ⁵								
9	10	11	12	13	14	15	16	
175	130	200	185	195	110	240	140	Length of spec.
225	160				210			PCR product(s)
430	430	430	515	430	430	430	430	Length of int.
								pos. control ¹
13(125)	38(199)	13(125)	37(196)	108(409)	13(125)	13(125)	37(196)	5'-primer(s) ²
5' -gTA 3'	5' -ACT 3'	5' -gTA 3'	5' -AgA 3'	5' -AgA 3'	5' -gTA 3'	5' -gTA 3'	5' -AgA 3'	
58(260)	67(286)	67(286)	85(341)	160(565)	37(196)	78(319)	70(295)	3'-primer(s) ³
5' -CCT 3'	5' -gAT 3'	5' -gAT 3'	5' -CAg 3'	5' -CAT 3'	5' -gTA 3'	5' -CAC 3'	5' -CTg 3'	
74(307)	78(319)				70(295)	79(323)		
5' -CAg 3'	5' -CAC 3'				5' -gTT 3'	5' -TgC 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
	10					15		*0112
9								*0113
					14			*0114
		11	12					*0202
		11						*0203
9	10	11	12	13	14	15	16	Well No.

Length of spec.	255	210	225	100	150	145	150	215
PCR product(s)								
Well No.	1	2	3	4	5	6	7	8
*0204		2						8
*0205			3					
DRB5 allele ⁴								
Well No.	1	2	3	4	5	6	7	8
DRB1*0907	1	2	3					
Well No.	1	2	3	4	5	6	7	8

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

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

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Lot-specific information

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175	130	200	185	195	110	240	140	Length of spec.
225	160				210			PCR product(s)
9	10	11	12	13	14	15	16	Well No.
			12					*0204
			12				16	*0205
								DRB5 allele⁴
9	10	11	12	13	14	15	16	Well No.
						15		DRB1*0907
9	10	11	12	13	14	15	16	Well No.

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

⁵Primer mix 9: Specific PCR fragment of 175 bp in the DRB5*0113 allele. Specific PCR fragment of 225 bp in the DRB5*0104 allele.

¹⁰Primer mix 10: Specific PCR fragment of 130 bp in the DRB5*0107 allele. Specific PCR fragment of 160 bp in the DRB5*0112 allele.

¹¹Primer mix 14: Specific PCR fragment of 110 bp in the DRB5*0114 allele. Specific PCR fragment of 210 bp in the DRB5*0109 allele.

[illegible]

CERTIFICATE OF ANALYSIS

Olerup SSP® DRB5 SSP

Product number:	101.123-24u/06u – without <i>Taq</i> pol.
Lot number:	88G
Expiry date:	2012-February-01
Number of tests:	24 test – Product No. 101.123-24u
	6 tests – Product No. 101.123-06u
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	2010-700-10
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	2010-700-14
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 or two additional 3'-primer(s). In primer solutions 14 and 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: 2010-February-19

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DRB5
Product number: 101.123-24u/06u
Lot number: 88G

Intended use: DRB5 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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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 9001:2008 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
2010-February-19

Olle Olerup
Managing Director

Lot No.: **88G**

Lot-specific information

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