

Lot No.: **21F**

Lot-specific Information

Olerup SSP[®] DRB4

Product number: 101.122-24u/06u – without *Taq* pol.
Lot number: 21F
Expiry date: 2010-October-01
Number of tests: 24 test – Product No. 101.122-24u
6 tests – Product No. 101.122-06u
Number of wells per test: 12
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. 21F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP[®] DRB4* LOT

The DRB4 specificity and interpretation tables have been updated for the DRB alleles described since the previous *Olerup SSP[®] DRB4* lot was made (**Lot No. Y01**).

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

Well	5'-primer	3'-primer	rationale
1	-	Modified	Increased yield of specific PCR product.
8	-	Modified	Increased specificity of specific primer pair.

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PRODUCT DESCRIPTION

DRB4 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB4*01010101 to DRB4*0301N alleles.

PLATE LAYOUT

Each test consists of 12 PCR reactions in a 16 well cut PCR plate. Wells 13 to 16 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	empty	empty	empty	empty

The 16 well cut PCR plate is marked with 'DRB4' in silver/gray ink.

Well No. 1 is marked with the Lot No. '21F'.

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 DRB4 alleles will be amplified by the primers in the DRB4 SSP subtyping kit. Thus, the interpretation of DRB4 SSP subtypings is not influenced by alleles of other DRB genes.

UNIQUELY IDENTIFIED ALLELES

All the DRB4 alleles, i.e. **DRB4*01010101 to DRB4*0301N**, recognized by the HLA Nomenclature Committee in October 2008¹ will give rise to unique amplification patterns by the primers in the DRB4 subtyping kit.

The DRB4 subtyping kit cannot distinguish the DRB4*01030101, DRB4*010302 to DRB4*010304 alleles.

²DRB4 alleles listed on the IMGT/HLA web page 2008-October-10, release 2.23.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOSES

The 10 phenotypically different DRB4 alleles can be combined in 55 homozygous and heterozygous combinations. Ten of these genotypes do not give rise to unique amplification patterns.

+--+---++ ---- 0101,0103 = 0103,0301N
+--+---+ +--- 0101,0105 = 0105,0301N
+--+---+ --+- 0101,0106 = 0106,0301N
+--+---+ ---- 0101,0107 = 0107,0301N
+--+---+ ---- 0101,0101 = 0101,0301N

0103 = 01030101, 010302, 010303 and 010304

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SPECIFICITY TABLE

DRB4 SSP subtyping

Specificities and sizes of the PCR products of the 12 primer mixes used for DRB4 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB4 alleles
1	185 bp	515 bp	01010101, 01030101, 01030102N, 010302, 010303, 010304, 0105, 0106, 0107
2	140 bp	430 bp	0102
3³	130 bp	430 bp	01010101, 0104 [?] , 0105 [?] , 0106, 0107 [?] , 0201N, 0301N
4	245 bp	515 bp	01010101, 0102, 01030101, 010302, 010303, 010304, 0104, 0105 [?] , 0106, 0107, 0201N
5⁴	150 bp	430 bp	01030102N
6	190 bp	430 bp	0104
7⁴	155 bp	430 bp	0102, 01030101, 01030102N, 010302, 010303, 010304, 0104 [?] , 0105 [?] , 0107 [?]
8	290 bp	515 bp	01010101, 0104 [?] , 0105 [?] , 0106 [?] , 0107 [?] , 0201N [?] , 0301N
9	155 bp	515 bp	0105
10	80 bp	515 bp	0201N
11	110 bp	430 bp	0106
12	210 bp	430 bp	0107

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 DRB4 SSP typings.

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

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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 DRB4 subtyping.

In addition, wells number 4, 8, 9 and 10 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.

³Primer mix 3 may yield somewhat less specific PCR product than the other DRB4 primer mixes.

⁴The DRB1*150101 to DRB1*1522 and DRB1*160101 to DRB1*160502 and DRB1*1607 to DRB1*1611 alleles might be faintly amplified by primer mix 7.

'?' The nucleotide sequences of the 3rd exon of the DRB4*0104, DRB4*0105 and DRB4*0107 alleles are not yet available. Thus, it is not known whether the DRB4*0104, DRB4*0105 and DRB4*0107 alleles will be amplified by primer mix 3 or 7. The complete 2nd exon nucleotide sequence of the DRB4*0105 allele is not known. Thus, it is not known whether the DRB4*0105 allele will be amplified by primer mix 4 or not. Second intron sequences of the DRB4*0104 to DRB4*0107 and the DRB4*0201N alleles is not known. Thus, it is not known whether these alleles will be amplified by primer mix 8 or not.

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INTERPRETATION TABLE								
DRB4 SSP subtyping								
Amplification patterns of the DRB4 alleles								
	Well							
	1	2	3	4	5	6	7⁸	8
Length of spec.	185	140	130	245	150	190	155	290
PCR product								
Length of int.	515	430	430	515	430	430	430	515
pos. control¹								
5'-primer²	28	42	105⁴	1st i⁵	1st i⁷	28	96	2nd i⁹
	5'-g AT ^{3'}	5'-AgT ^{3'}	5'-A AA ^{3'}	5'-ggg ^{3'}	5'-CAA ^{3'}	5'-g AT ^{3'}	5'-CAA ^{3'}	5'-TgA ^{3'}
3'-primer³	76	76	135⁴	5⁶	42	77	135⁴	2nd i⁹
	5'-T gT ^{3'}	5'-T gC ^{3'}	5'-gCT ^{3'}	5'-Tg C ^{3'}	5'-TC A ^{3'}	5'-A gT ^{3'}	5'-gCC ^{3'}	5'-TTC ^{3'}
Well No.	1	2	3	4	5	6	7⁸	8
DRB4 allele								
*01010101	1		3	4				8
*0102		2		4			7	
*01030101, 010302-010304	1			4			7	
*01030102N¹⁰	1				5		7	
*0104			?	4		6	?	?
*0105	1		?	?			?	?
*0106	1		3	4				?
*0107	1		?	4			?	?
*0201N			3	4				?
*0301N			3					8
DRB4 allele								
Well No.	1	2	3	4	5	6	7⁸	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 DRB4 subtyping.

In addition, wells number 4, 8, 9 and 10 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 unless otherwise noted, 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 unless otherwise noted, 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.

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INTERPRETATION TABLE				
DRB4 SSP subtyping				
Amplification patterns of the DRB4 alleles				
Well				
9	10	11	12	
155	80	110	210	Length of spec.
				PCR product
515	515	430	430	Length of int.
				pos. control ¹
42	28	112	28	5'-primer ²
5'-AgT ^{3'}	5'-g AT ^{3'}	5'-AC T ^{3'}	5'-g AT ^{3'}	
81	42	135 ⁴	84	3'-primer ³
5'-gTg ^{3'}	5'-TC A ^{3'}	5'-gCT ^{3'}	5'-CCg ^{3'}	
9	10	11	12	Well No.
				DRB4 allele
				*01010101
				*0102
				*01030101, 010302- 010304
				*01030102N ¹⁰
				*0104
9				*0105
		11		*0106
			12	*0107
	10			*0201N
				*0301N
				DRB4 allele
9	10	11	12	Well No.

⁴Matching sequences within the 3rd exon.

⁵Matching sequences within the 1st intron.

⁶Matching sequences from the 3'-end of the 1st intron into the 5'-end of the 2nd exon.

⁷Matching the sequence of the 3'-end of the 1st intron.

⁸The DRB1*150101 to DRB1*1531 and DRB1*160101 to DRB1*1605 and DRB1*1607 to DRB1*1613N alleles might be faintly amplified by primer mix 7.

⁹Matching sequences within the 2nd intron.

¹⁰The DRB4*01010102N allele has been renamed to DRB4*01030102N.

'?' The nucleotide sequences of the 3rd exon of the DRB4*0104, DRB4*0105 and DRB4*0107 alleles are not yet available. Thus, it is not known whether the DRB4*0104, DRB4*0105 and DRB4*0107 alleles will be amplified by primer mix 3 or 7. The complete 2nd exon nucleotide sequence of the DRB4*0105 allele is not known. Thus, it is not known whether the DRB4*0105 allele will be amplified by primer mix 4 or not. Second intron sequences of the DRB4*0104 to DRB4*0107 and the DRB4*0201N alleles is not known. Thus, it is not known whether these alleles will be amplified by primer mix 8 or not.

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CERTIFICATE OF ANALYSIS

Olerup SSP® DRB4 SSP

Product number: 101.122-24u/06u – without *Taq* pol.
Lot number: 21F
Expiry date: 2010-October-01
Number of tests: 24 test – Product No. 101.122-24u
6 tests – Product No. 101.122-06u
Number of wells per test: 12

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2008-524-01	9	2007-357-09
2	2007-357-02	10	2007-357-10
3	2007-357-03	11	2007-357-11
4	2007-357-04	12	2007-357-12
5	2007-357-07		
6	2007-357-06		
7	2007-357-07		
8	2008-524-08		

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 No. 6 and 9 to 12 were available. The specificities of the primers in primer solutions 6 and 9 were tested by separately adding one additional 5'-primer, respectively, one additional 3'-primer. In primer solutions 10 and 11 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solution 12 it was only possible to test the 5'-primer, the 3'-primer was not possible to test.

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

Date of approval: 2009-May-25

Approved by:

Quality Control, Supervisor

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Declaration of Conformity

Product name: *Olerup* SSP® DRB4
Product number: 101.122-24u/06u
Lot number: 21F

Intended use: DRB4 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 9001:2000 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

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