Olerup SSP® DRB4

Product number:	**101.122-24/06 – including *Taq* pol.**
Lot number:	**45G**
Expiry date:	**2011-October-01**
Number of tests:	**24 test – Product No. 101.122-24**
	6 tests – Product No. 101.122-06
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. 45G.

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. 21F)**.

The **DRB4** primer set is unchanged compared to the previous lot.

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. '45G'.
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 DRB4 alleles will be amplified by the primers in the DRB4 SSP subtyping kit[1]. Thus, the interpretation of DRB4 SSP subtypings is not influenced by alleles of other DRB genes.
[1]The DRB1*15010101 to DRB1*1541 and DRB1*160101 to DRB1*160502 and DRB1*1607 to DRB1*1615 alleles might be faintly amplified by primer mix 7.

UNIQUELY IDENTIFIED ALLELES
All the DRB4 alleles, i.e. **DRB4*01010101 to DRB4*0301N**, recognized by the HLA Nomenclature Committee in October 2009[1] 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.

[2]DRB4 alleles listed on the IMGT/HLA web page 2009-October-19, release 2.27.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES
The 10 phenotypically different DRB4 alleles can be combined in 55 homozygous and heterozygous combinations. Twenty-one of these genotypes do not give rise to unique amplification patterns.

```
+-++-+++ ----    0101,0104 = 0103,0104
+-++--++ +---    0101,0105 = 0103,0105 = 0105,0105 = 0105,0301N
+-++--++ ---+    0101,0107 = 0103,0107 = 0107,0107 = 0107,0301N
+-++--++ ----    0101,0103 = 0103,0301N
+-++---+ --+-    0101,0106 = 0106,0106 = 0106,0301N
+-++---+ ----    0101,0101 = 0101,0301N
--++-+++ ----    0104,0104 = 0104,0301N
--++---+ -+--    0201N,0201N = 0201N,0301N

0103 = 01030101 and 010302 to 010304
```

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, 01030101-010304, 0105-0107
2	140 bp	430 bp	*0102
3[3]	130 bp	430 bp	*01010101, 0104?, 0105?, 0106, 0107?, 0201N, 0301N
4	245 bp	**515 bp**	*01010101-01030101, 010302-0104, 0105?, 0106, 0107, 0201N
5[4]	155 bp	430 bp	*01030102N
6	190 bp	430 bp	*0104
7[5]	155 bp	430 bp	*0102-010304, 0104?, 0105?, 0107?
8	290 bp	**515 bp**	*01010101, 0104?-0107?, 0201N?, 0301N
9[6]	155 bp	**515 bp**	*0105
10	85 bp	**515 bp**	*0201N
11	110 bp	430 bp	*0106
12	210 bp	430 bp	*0107

[1]Alleles 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 lengths 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 inherit 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.
[2]The 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.

In the presence of a specific amplification the intensity of the control band often decreases.
[3]Primer mix 3 may yield somewhat less specific PCR product than the other DRB4 primer mixes.
[4]Primer mix 5 may have tendencies of unspecific amplifications.
[5]The DRB1*15010101 to DRB1*1541 and DRB1*160101 to DRB1*160502 and DRB1*1607 to DRB1*1615 alleles might be faintly amplified by primer mix 7.
[6]Primer mix 9 might have tendencies of primer dimer formation.
'?' 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.

INTERPRETATION TABLE

DRB4 SSP subtyping

Amplification patterns of the DRB4 alleles

	Well							
	1	2	3	4	5	6	7 [8]	8
Length of spec. PCR product	185	140	130	245	155	190	155	290
Length of int. pos. control[1]	515	430	430	515	430	430	430	515
5'-primer[2]	28(170)	42(213)	105(401)[4]	1st I[5]	1st I[7]	28(170)	96(375)	2nd I[9]
	5' -gAT 3'	5' -AgT 3'	5' -AAA 3'	5' -ggg 3'	5' -CAA 3'	5' -gAT 3'	5' -CAA 3'	5' -TgA 3'
3'-primer[3]	76(314)	76(314)	135(490)[4]	5 [6]	42(213)	77(317)	135(490)[4]	2nd I[9]
	5' -TgT 3'	5' -TgC 3'	5' -gCT 3'	5' -TgC 3'	5' -TCA 3'	5' -AgT 3'	5' -gCC 3'	5' -TTC 3'
Well No.	1	2	3	4	5	6	7 [8]	8
DRB4 allele								
***01010101**	1		3	4				8
***0102**		2		4			7	
***01030101, 010302-010304**	1			4			7	
***01030102N**	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	7 [8]	8

[1]The 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.
[2]The codon, and in parenthesis the nucleotide, in the 2nd or 3rd exon unless otherwise noted, 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.
[3]The codon, and in parenthesis the nucleotide, 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 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.

INTERPRETATION TABLE				
DRB4 SSP subtyping				
Amplification patterns of the DRB4 alleles				
Well				
9	10	11	12	
155	85	110	210	Length of spec. PCR product
515	515	430	430	Length of int. pos. control[1]
42(213)	28(170)	112(421)[4]	28(170)	5'-primer[2]
5' -AgT 3'	5' -gAT 3'	5' -ACT 3'	5' -gAT 3'	
81(328)	42(213)	135(490)[4]	84(337)	3'-primer[3]
5' -gTg 3'	5' -TCA 3'	5' -gCT 3'	5' -CCg 3'	
9	10	11	12	Well No.
				DRB4 allele
				*01010101
				*0102
				*01030101, 010302-010304
				*01030102N
				*0104
9				*0105
		11		*0106
			12	*0107
	10			*0201N
				*0301N
				DRB4 allele
9	10	11	12	Well No.

[4]Matching sequences within the 3rd exon.
[5]Matching sequences within the 1st intron.
[6]Matching sequences from the 3'-end of the 1st intron into the 5'-end of the 2nd exon.
[7]Matching the sequence of the 3'-end of the 1st intron.
[8]The DRB1*15010101 to DRB1*1541 and DRB1*160101 to DRB1*160502 and DRB1*1607 to DRB1*1615 alleles might be faintly amplified by primer mix 7.
[9]Matching sequences within the 2nd intron.
[10]The 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.

CELL LINE VALIDATION SHEET

DRB4 SSP kit

					Well											
					1	2	3	4	5	6	7	8	9	10	11	12
				Prod. No.:	200965301	200735702	200735703	200735704	200735705	200735706	200735707	200852408	200965309	200735710	200735711	200735712
	IHWC cell line		DRB4													
1	9001	SA			-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*0103		+	-	-	+	-	-	+	-	-	-	-	-
3	9011	E4181324			-	-	-	-	-	-	f	-	-	-	-	-
4	9275	GU373			-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011			-	-	-	-	-	-	f	-	-	-	-	-
6	9353	SM	*0103		+	-	-	+	-	-	+	-	-	-	-	-
7	9020	QBL			-	-	-	-	-	-	-	-	-	-	-	-
8	9025	DEU	*0101		+	-	+	+	-	-	-	+	-	-	-	-
9	9026	YAR	*0103		+	-	-	+	-	-	+	-	-	-	-	-
10	9107	LKT3	*0103		+	-	-	+	-	-	+	-	-	-	-	-
11	9051	PITOUT	*0101		+	-	+	+	-	-	-	+	-	-	-	-
12	9052	DBB	*0103N		+	-	-	-	+	-	+	-	-	-	-	-
13	9004	JESTHOM			-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA			-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*0103		+	-	-	+	-	-	+	-	-	-	-	-
16	9037	SWEIG007			-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540			-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*0101		+	-	+	+	-	-	-	+	-	-	-	-
19	9038	BM16			-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005			-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA			-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE			-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL			-	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH			-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*0101		+	-	+	+	-	-	-	+	-	-	-	-
26	9285	WT49			-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*0103		+	-	-	+	-	-	+	-	-	-	-	-
28	9320	BEL5GB	*0101		+	-	+	+	-	-	+	+	-	-	-	-
29	9050	MOU	*0101		+	-	+	+	-	-	-	+	-	-	-	-
30	9021	RSH			-	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF			-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG			-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*0103		+	-	-	+	-	-	+	-	-	-	-	-
34	9104	DHIF			-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*0103		+	-	-	+	-	-	+	-	-	-	-	-
36	9024	KT17	*0103		+	-	-	+	-	-	+	-	-	-	-	-
37	9065	HHKB			-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL			-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*0102		-	+	-	+	-	-	+	-	-	-	-	-
40	9134	WHONP199	*0103		+	-	-	+	-	-	+	-	-	-	-	-
41	9055	H0301			-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089			-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*0103		+	-	-	+	-	-	+	-	-	-	-	-
44	9057	TEM			-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*0101	*0103	+	-	+	+	-	-	+	+	-	-	-	-
46	9013	SCHU			-	-	-	-	-	-	f	-	-	-	-	-
47	9045	TUBO			-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND			-	-	-	-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® DRB4 SSP

Product number: **101.122-24/06 – including *Taq* pol.**
Lot number: **45G**
Expiry date: **2011-October-01**
Number of tests: **24 test – Product No. 101.122-24**
6 tests – Product No. 101.122-06
Number of wells per test: **12**

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2009-653-01	9	2009-653-09
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	2007-357-05		
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 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-November-09

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DRB4
Product number: 101.122-24/06
Lot number: 45G

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 9001:2008 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
2009-November-09

Olle Olerup

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