

Olerup SSP® DRB1*07

Product numbers:	101.118-24 – including <i>Taq</i> polymerase
Lot number:	67F
Expiry date:	2011-April-01
Number of tests:	24
Number of wells per test:	14
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. 67F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*® DRB1*07 LOT

The DRB1*07 specificity and interpretation tables have been updated for the DRB1 alleles described since the previous *Olerup SSP*® DRB1*07 lot (**Lot No. 32E**) was made.

Two wells have been added to the DRB1*07 kit,
wells **13** and **14**.

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

Well	5'-primer	3'-primer	rationale
4	-	Modified	Increased specificity of specific primer pair, exchanged positive control primer pair.
13	New	New	Primer pair for the DRB1*0716 allele.
14	New	New	Primer pair for the DRB1*0717 allele.

PRODUCT DESCRIPTION

DRB1*07 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*0701 to DRB1*0717 alleles.

PLATE LAYOUT

Each test consists of 14 PCR reactions in a 16 well cut PCR plate. Wells 15 and 16 are empty.

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

The 16 well cut PCR plate is marked with 'DRB1*07' in silver/gray ink.

Well No. 1 is marked with the Lot No. '67F'.

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

The interpretation of DRB1*07 PCR-SSP subtypings will only be influenced the DRB1*0908 allele, when present on the other haplotype. Thus, the interpretation of DRB1*07 subtypings is only marginally influenced by other groups of DRB1 alleles.

UNIQUELY IDENTIFIED ALLELES

All the DRB1*07 alleles, i.e. **DRB1*0701 to DRB1*0717**, recognized by the HLA Nomenclature Committee in April 2009¹ will give rise to unique amplification patterns by the primers in the DRB1*07 subtyping kit.

The DRB1*07 subtyping kit cannot distinguish the DRB1*07010101 to DRB1*070103 alleles.

¹DRB1 alleles listed on the IMGT/HLA web page 2009-April-17, release 2.25.2, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 16 DRB1*07 alleles can be combined in 136 homozygous and heterozygous combinations. Ninety-three of these genotypes do not give rise to unique amplification patterns. The different sizes of the specific PCR products generated by primer mixes 4 and 5 have not been considered in this calculation.

```
++++----- 0703,0710N = 0704,0709 = 0709,0710N
+++-+---- 0703,0705 = 0705,0709 = 0709,0711
+++--+--- 0703,0706 = 0706,0709
+++---+ 0703,0707 = 0707,0709
+++-----+ 0703,0708 = 0708,0709
+++----- +----- 0703,0712 = 0709,0712
+++----- -+----- 0703,0713 = 0709,0713
+++----- --+----- 0703,0714 = 0709,0714
+++----- ---+----- 0703,0715 = 0709,0715
+++----- ----+----- 0703,0716 = 0709,0716
+++----- -----+ 0703,0717 = 0709,0717
+++----- 0701,0703 = 0701,0709 = 0703,0709 = 0709,0709
++-++---- 0704,0705 = 0705,0710N = 0710N,0711
++-+-+--- 0704,0706 = 0706,0710N
++-+--+ 0704,0707 = 0707,0710N
++-+----+ 0704,0708 = 0708,0710N
++-+----- +----- 0704,0712 = 0710N,0712
++-+----- -+----- 0704,0713 = 0710N,0713
++-+----- --+----- 0704,0714 = 0710N,0714
++-+----- ---+----- 0704,0715 = 0710N,0715
++-+----- ----+----- 0704,0716 = 0710N,0716
++-+----- -----+ 0704,0717 = 0710N,0717
++-+----- 0701,0704 = 0701,0710N = 0704,0710N =
0710N,0710N
++-++---- 0705,0706 = 0706,0711
++-+-+--- 0705,0707 = 0707,0711
++-+--+ 0705,0708 = 0708,0711
++-+----+ 0705,0712 = 0711,0712
++-+----- -+----- 0705,0713 = 0711,0713
++-+----- --+----- 0705,0714 = 0711,0714
++-+----- ---+----- 0705,0715 = 0711,0715
++-+----- ----+----- 0705,0716 = 0711,0716
++-+----- -----+ 0705,0717 = 0711,0717
++-+----- 0701,0705 = 0701,0711 = 0705,0705 = 0705,0711
++-+----- 0701,0706 = 0706,0706
++-+-----+ 0701,0707 = 0707,0707
++-+-----+ 0701,0708 = 0708,0708
++-+----- +----- 0701,0712 = 0712,0712
++-+----- -+----- 0701,0713 = 0713,0713
++-+----- --+----- 0701,0714 = 0714,0714
++-+----- ---+----- 0701,0715 = 0715,0715
++-+----- ----+----- 0701,0716 = 0716,0716
++-+----- -----+ 0701,0717 = 0717,0717
```

0701 = 07010101-070103

SPECIFICITY TABLE

DRB1*07 SSP subtyping

Specificities and sizes of the PCR products of the 14 primer mixes used for DRB1*07 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB1*07 alleles	Amplified non-DRB1*07 alleles ³
1⁵	235 bp	515 bp	07010101-070103, 0703-0716	
2	185 bp	430 bp	07010101-070103, 0705-0710N, 0712-0716	
3	185 bp	430 bp	0703, 0709	
4^{4,6}	90, 230 bp	515 bp	0704, 0710N	
5⁷	190, 230 bp	430 bp	0705, 0711	
6	170 bp	430 bp	0706	
7	165 bp	430 bp	0707	
8	165 bp	430 bp	0708	
9^{3,4,5}	105 bp	430 bp	0712	0908
10	165 bp	515 bp	0713	
11⁴	105 bp	515 bp	0714	
12	210 bp	430 bp	0715	
13	205 bp	430 bp	0716	
14	215 bp	430 bp	0717	

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*07 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 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 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

Lot No.: 67F

Lot-specific information

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band in order to help in the correct orientation of the DRB1*01 subtyping.

In addition, wells number 4, 10 and 11 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.

³Due to sharing of sequence motifs, the DRB1*0908 allele is amplified by primer mix 9.

⁴Specific PCR fragments shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR bands.

⁵Primer mixes 1 and 9 may have tendencies of giving rise to primer dimer artefacts.

⁶Primer mix 4: Specific PCR fragment of 90 bp in the DRB1*0710N allele. Specific PCR fragment of 230 bp in the DRB1*0704 allele.

⁷Primer mix 5: Specific PCR fragment of 190 bp in the DRB1*0705 allele. Specific PCR fragment of 230 bp in the DRB1*0711 allele.

INTERPRETATION TABLE														
DRB1*07 PCR-SSP subtyping														
Amplification patterns of the DRB1*0701 to *0717 alleles														
	Well ⁵													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Length of spec.	235	185	185	90	190	170	165	165	105	165	105	210	205	215
PCR product				230	230									
Length of int.	515	430	430	515	430	430	430	430	430	515	515	430	430	430
pos. control ¹														
5'-primer(s) ²	14	29	29	14	14	14	14	37	57	14	14	21	23	14
	5'-AT A ^{3'}	5'-AgA ^{3'}	5'-AgT ^{3'}	5'-AT A ^{3'}	5'-AT A ^{3'}	5'-AT A ^{3'}	5'-AT A ^{3'}	5'-Ag g ^{3'}	5'-CT A ^{3'}	5'-AT A ^{3'}	5'-AT A ^{3'}	5'-g AA ^{3'}	5'-Ag g ^{3'}	5'-AT A ^{3'}
	14		30											
	5'-AT A ^{3'}		5'-gA T ^{3'}											
3'-primer(s) ³	78	78	78	29	64	57	55	78	78	55	35	78	78	72
	5'-CAC ^{3'}	5'-CAC ^{3'}	5'-CAC ^{3'}	5'-..T ^{3'}	5'-T CC ^{3'}	5'-C Ag ^{3'}	5'-CCA ^{3'}	5'-CAC ^{3'}	5'-CAC ^{3'}	5'-g CA ^{3'}	5'-C CC ^{3'}	5'-CAC ^{3'}	5'-CAC ^{3'}	5'-CCA ^{3'}
	78			77	77									
	5'-gTA ^{3'}			5'-A AT ^{3'}	5'-A gg ^{3'}									
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
DRB1 allele ⁴														
*07010101-070103	1	2												
*0703	1		3											
*0704	1			4										
*0705	1	2			5									
*0706	1	2				6								
*0707	1	2					7							
*0708	1	2						8						
*0709	1	2	3											
*0710N	1	2		4										
*0711	1				5									
*0712	1	2							9					
*0713	1	2								10				
*0714	1	2									11			
*0715	1	2										12		
*0716	1	2											13	
*0717	1	2												14
*0908									9					
DRB1 allele ⁴														
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14

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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*07 subtyping.

In addition, wells number 4, 10 and 11 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 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.

⁴The DRB1*0702 allele has been shown to be identical to DRB1*0701.

⁵Primer mix 4: Specific PCR fragment of 90 bp in the DRB1*0710N allele. Specific PCR fragment of 230 bp in the DRB1*0704 allele.

Primer mix 5: Specific PCR fragment of 190 bp in the DRB1*0705 allele. Specific PCR fragment of 230 bp in the DRB1*0711 allele.

CELL LINE VALIDATION SHEET																		
DRB1*07 SSP subtyping kit																		
					Well													
					1	2	3	4	5	6	7	8	9	10	11	12	13	14
				Production No.	200957401	200957402	200843703	200957404	200957405	200957406	200957407	200843708	200843709	200843710	200843711	200843712	200957413	200957414
	IHC cell line		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	9004	JESTHOM	*0101		-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*0802		-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*0901		-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*1101		-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*0301	*1301	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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		-	-	-	-	-	-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® DRB1*07 SSP

Product numbers: 101.118-24 – including *Taq* polymerase
Lot number: 67F
Expiry date: 2011-April-01
Number of tests: 24
Number of wells per test: 14

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2009-574-01	9	2008-437-09
2	2009-574-02	10	2008-437-10
3	2008-437-03	11	2008-437-11
4	2009-574-04	12	2008-437-12
5	2009-574-05	13	2009-574-13
6	2009-574-06	14	2009-574-14
7	2009-574-07		
8	2008-437-08		

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 3 to 14 were available. The specificities of the primers in primer solutions 4, 5, 6 and 9 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 3, 8, 12 and 13 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 5, 7, 10, 11 and 14 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 4 and 5, one 3'-primer was not possible to test. In addition, one 3'-primer in primer solution 1 was 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

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*07
Product number: 101.118-24
Lot number: 67F

Intended use: DRB1*07 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.: **67F**

Lot-specific information

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