Olerup SSP® DRB1*09

Product number:	**101.120-06 – including *Taq* polymerase**
Lot number:	**07G**
Expiry date:	**2011-July-01**
Number of tests:	**6**
Number of wells per test:	**8**
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. 07G.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® DRB1*09 LOT

The DRB1*09 specificity and interpretation tables have been updated for the DRB alleles described since the previous *Olerup* SSP® DRB1*09 lot was made **(Lot No. 18F)**.

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

Well	5'-primer	3'-primer	rationale
3	Exchanged	Exchanged	New primer pair for the DRB1*090201 and *090202 alleles.
5	-	Added	New primer added for the DRB1*0908 allele.

PRODUCT DESCRIPTION

DRB1*09 SSP subtyping

CONTENT
The primer set contains 5'- and 3'-primers for identifying the DRB1*0901 to DRB1*0908 alleles.

PLATE LAYOUT
Each test consists of 8 PCR reactions in an 8 well PCR plate.

1	2	3	4	5	6	7	8

The 8 well PCR plate is marked with 'DR9' in silver/gray ink.
Well No. 1 is marked with the Lot No. '07G'.
The PCR plates are covered with a PCR-compatible foil.
Please note: When removing each 8 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*09 alleles will be amplified by the DRB1*09 subtyping kit. Thus, the interpretation of DRB1*09 subtypings is not influenced by other groups of DRB1 alleles or other DRB genes.

UNIQUELY IDENTIFIED ALLELES
All the DRB1*09 alleles, i.e. **DRB1*0901 to DRB1*0908**, recognized by the HLA Nomenclature Committee in May 2009[1] will give rise to unique amplification patterns by the primers in the DRB1*09 subtyping kit.
The DRB1*09 SSP subtyping kit cannot separate the DRB1*090102 to DRB1*090104 or the DRB1*090201 and DRB1*090202 alleles.

[1]DRB alleles listed on the IMGT/HLA web page 2009-May-11, release 2.25.2, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES
The 8 DRB1*09 alleles can be combined in 36 homozygous and heterozygous combinations. Eighteen of these genotypes do not give rise to unique amplification patterns.

```
++-++---   0903,0904 = 0903,0908
++-+----   0901,0903 = 0903,0903
++--++--   0904,0905 = 0905,0908
++--+-+-   0904,0906 = 0906,0908
++--+--+   0904,0907 = 0907,0908
++--+---   0901,0904 = 0901,0908 = 0904,0904 = 0904,0908
++----+-   0901,0906 = 0906,0906
++-----+   0901,0907 = 0907,0907

0901 = 090102-090104, 0902 = 090201-090202
```

SPECIFICITY TABLE

DRB1*09 SSP subtyping

Specificities and sizes of the PCR products of the 8 primer mixes used for DRB1*09 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified DRB1*09 alleles
1	195 bp	**515 bp**	090102-0904, 0906-0908
2[3]	130 bp	430 bp	090102-090104, 0903-0907
3	185 bp	430 bp	090201, 090202
4	215 bp	430 bp	0903
5[4]	185, 215 bp	430 bp	0904, 0908
6	250 bp	430 bp	0905
7	220 bp	430 bp	0906
8	175 bp	430 bp	0907

[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 DRB1*09 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 DRB1*09 subtyping.
In the presence of a specific amplification the intensity of the control band often decreases.
[3]Specific PCR fragments shorter than 150 base pairs have a lower intensity than longer PCR bands.
[4]Primer mix 5: Specific PCR fragment of 185 bp in the DRB1*0908 allele. Specific PCR fragment of 215 bp in the DRB1*0904 allele.

INTERPRETATION TABLE

DRB1*09 SSP subtyping

Amplification patterns of the DRB1*09 alleles

	Well[5]							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Length of spec.	**195**	**130**	**185**	**215**	**185**	**250**	**220**	**175**
PCR product					**215**			
Length of int.	**515**	**430**	**430**	**430**	**430**	**430**	**430**	**430**
pos. control[1]								
5'-primer[2]	**26(164)**	**28(170)**	**9(112)**	**9(112)**	**9(112)**	**9(112)**	**26(165)**	**13(125)**
	5' -gTA 3'	**5' -gCA 3'**	**5' -TgA 3'**	**5' -TgA 3'**	**5' -TgA 3'**	**5' -TgA 3'**	**5' -TAT 3'**	**5' -gTA 3'**
3'-primer(s)[3]	**78(319)**	**57(257)**	**57(257)**	**67(286)**	**57(256)**	**78(319)**	**86(344)**	**57(257)**
	5' -CAC 3'	**5' -CgA 3'**	**5' -CAT 3'**	**5' -gAA 3'**	**5' -gCT 3'**	**5' -gTA 3'**	**5' -CCA 3'**	**5' -CgA 3'**
			57(258)		**67(286)**			
			5' -gCg 3'		**5' -gAg 3'**			
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
DRB1 allele[4]								
***090102-090104**	**1**	**2**						
***090201, 090202**	**1**		**3**					
***0903**	**1**	**2**		**4**				
***0904**	**1**	**2**			**5**			
***0905**		**2**				**6**		
***0906**	**1**	**2**					**7**	
***0907**	**1**	**2**						**8**
***0908**	**1**				**5**			
DRB1 allele[4]								
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**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 DRB1*09 subtyping.
In the presence of a specific amplification the intensity of the control band often decreases.
[2]The codon, and in parenthesis the nucleotide, 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 shown.
[3]The codon, and in parenthesis the nucleotide, in the 2nd and 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 shown.
[4]The nucleotide sequence of the DRB1*090101 allele has been shown to contain errors and be identical to DRB1*090102.
[5]Primer mix 5: Specific PCR fragment of 185 bp in the DRB1*0908 allele. Specific PCR fragment of 215 bp in the DRB1*0904 allele.

CELL LINE VALIDATION SHEET

DRB1*09 SSP subtyping kit

					Well							
					1	2	3	4	5	6	7	8
				Production No.	200616901	200616902	200961603	200616904	200961605	200961606	200616907	200852208
	IHWC 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	9025	DEU	*0401		-	-	-	-	-	-	-	-
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*09 SSP

Product number: **101.120-06 – including *Taq* polymerase**
Lot number: **07G**
Expiry date: **2011-July-01**
Number of tests: **6**
Number of wells per test: **8**

Well specifications:

Well No.	Production No.
1	2006-169-01
2	2006-169-02
3	2009-616-03
4	2006-169-04
5	2009-616-05
6	2009-616-06
7	2006-169-07
8	2008-522-08

The specificity of each primer solution of the kit has been tested against 48 well characterized cell line DNAs.

No DNAs carrying the allele to be amplified by primer solutions 3 to 8 were available. The specificities of the primers in primer solutions 3 to 8 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer.

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

Date of approval: 2009-July-20

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*09
Product number: 101.120-06
Lot number: 07G

Intended use: DRB1*09 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-July-20

Olle Olerup
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