

Olerup SSP[®] DRB1*09

Product number:	101.120-06u – without <i>Taq</i> polymerase
Lot number:	60K
Expiry date:	2013-April-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. 60K.

**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. 07G**).

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

Well	5'-primer	3'-primer	rationale
6	-	Added	New primer added for the DRB1*09:09 allele.

PRODUCT DESCRIPTION

DRB1*09 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*09:01 to DRB1*09:09 alleles.

PLATE LAYOUT

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

1	2	3	4	5	6	7	8
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The 8 well PCR plate is marked with 'DR9' in silver/gray ink.

Well No. 1 is marked with the Lot No. '60K'.

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 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*09:01 to DRB1*09:09**, recognized by the HLA Nomenclature Committee in July 2010¹ 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*09:01:02 to DRB1*09:01:07 or the DRB1*09:02:01 and DRB1*09:02:02 alleles.

¹DRB alleles listed on the IMGT/HLA web page 2010-July-16, release 3.1.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 15 alleles generate 9 amplification patterns that can be combined in 45 homozygous and heterozygous combinations. 32 of these genotypes do not give rise to unique amplification patterns.

+++--+-	09:01:02, *09:05 = *09:02:01, *09:09
++-++--	*09:03, *09:04 = *09:03, *09:08
++-+-+-	*09:03, *09:05 = *09:03, *09:09
++-+---	*09:01:02, *09:03 = *09:03, *09:03
++-+++-	*09:04, *09:05 = *09:04, *09:09 = *09:05, *09:08 = *09:08, *09:09
++-+-+-	*09:04, *09:06 = *09:06, *09:08
++-+---+	*09:04, *09:07 = *09:07, *09:08
++-+---	*09:01:02, *09:04 = *09:01:02, *09:08 = *09:04, *09:04 = *09:04, *09:08
++-+++-	*09:05, *09:06 = *09:06, *09:09
++-+-+-	*09:05, *09:07 = *09:07, *09:09
++-+---	*09:01:02, *09:05 = *09:01:02, *09:09 = *09:05, *09:09 = *09:09, *09:09
++-+---+	*09:01:02, *09:06 = *09:06, *09:06
++-+---	*09:01:02, *09:07 = *09:07, *09:07

*09:01:02 = *09:01:02-09:01:07

*09:02:01 = *09:02:01-09:02:01

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	*09:01:02-09:04, 09:06-09:09
2³	130 bp	430 bp	*09:01:02-09:01:07, 09:03-09:07, 09:09
3	185 bp	430 bp	*09:02:01-09:02:02
4	215 bp	430 bp	*09:03
5⁴	185 bp, 215 bp	430 bp	*09:04, 09:08
6⁵	250 bp, 275 bp	430 bp	*09:05, 09:09
7	220 bp	430 bp	*09:06
8	175 bp	430 bp	*09:07

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*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 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 DRB1*09 subtyping.

In the presence of a specific amplification the intensity of the control band often decreases.

³Specific PCR fragments shorter than 150 base pairs have a lower intensity than longer PCR bands.

⁴Primer mix 5: Specific PCR fragment of 185 bp in the DRB1*09:08 allele. Specific PCR fragment of 215 bp in the DRB1*09:04 allele.

⁵Primer mix 6: Specific PCR fragment of 250 bp in the DRB1*09:05 allele. Specific PCR fragment of 275 bp in the DRB1*09:09 allele.

INTERPRETATION TABLE								
DRB1*09 SSP subtyping								
Amplification patterns of the DRB1*09 alleles								
	Well⁵							
	1	2	3	4	5	6	7	8
Length of spec.	195	130	185	215	185	250	220	175
PCR product					215	275		
Length of int.	515	430	430	430	430	430	430	430
pos. control¹								
5'-primer²	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)	87(348)		
			5' -gCg 3'		5' -gAg 3'	5' -CAT 3'		
Well No.	1	2	3	4	5	6	7	8
DRB1 allele⁴								
*09:01:02-09:01:07	1	2						
*09:02:01-09:02:02	1		3					
*09:03	1	2		4				
*09:04	1	2			5			
*09:05		2				6		
*09:06	1	2					7	
*09:07	1	2						8
*09:08	1				5			
*09:09	1	2				6		
DRB1 allele⁴								
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 DRB1*09 subtyping. In the presence of a specific amplification the intensity of the control band often decreases.

²The 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.

³The 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.

⁴The nucleotide sequence of the DRB1*090101 allele has been shown to contain errors and be identical to DRB1*09:01:02.

⁵Primer mix 5: Specific PCR fragment of 185 bp in the DRB1*09:08 allele. Specific PCR fragment of 215 bp in the DRB1*09:04 allele.

Primer mix 6: Specific PCR fragment of 250 bp in the DRB1*09:05 allele. Specific PCR fragment of 275 bp in the DRB1*09:09 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	201078106	200616907	200852208
	IHC cell line		DRB1										
1	9001	SA	*01:01		-	-	-	-	-	-	-	-	-
2	9280	LK707	*15:02	*04:05	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*15:02		-	-	-	-	-	-	-	-	-
4	9275	GU373	*03:01		-	-	-	-	-	-	-	-	-
5	9009	KAS011	*16:01		-	-	-	-	-	-	-	-	-
6	9353	SM	*04:07	*08:03	-	-	-	-	-	-	-	-	-
7	9020	QBL	*03:01		-	-	-	-	-	-	-	-	-
8	9025	DEU	*04:01		-	-	-	-	-	-	-	-	-
9	9026	YAR	*04:02		-	-	-	-	-	-	-	-	-
10	9107	LKT3	*04:05		-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*07:01		-	-	-	-	-	-	-	-	-
12	9052	DBB	*07:01		-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*01:01		-	-	-	-	-	-	-	-	-
14	9071	OLGA	*08:02		-	-	-	-	-	-	-	-	-
15	9075	DKB	*09:01		+	+	-	-	-	-	-	-	-
16	9037	SWEIG007	*11:01		-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*03:01	*13:01	-	-	-	-	-	-	-	-	-
18	9257	32367	*09:01	*11:01	+	+	-	-	-	-	-	-	-
19	9038	BM16	*12:01		-	-	-	-	-	-	-	-	-
20	9059	SLE005	*13:02		-	-	-	-	-	-	-	-	-
21	9064	AMALA	*14:02		-	-	-	-	-	-	-	-	-
22	9056	KOSE	*13:02	*14:54	-	-	-	-	-	-	-	-	-
23	9124	IHL	*08:03	*14:14	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*11:01		-	-	-	-	-	-	-	-	-
25	9049	IBW9	*07:01		-	-	-	-	-	-	-	-	-
26	9285	WT49	*03:01		-	-	-	-	-	-	-	-	-
27	9191	CH1007	*04:05	*10:01	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*04:16	*07:01	-	-	-	-	-	-	-	-	-
29	9050	MOU	*07:01		-	-	-	-	-	-	-	-	-
30	9021	RSH	*03:02		-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*03:01		-	-	-	-	-	-	-	-	-
32	9297	HAG	*13:03		-	-	-	-	-	-	-	-	-
33	9098	MT14B	*04:04		-	-	-	-	-	-	-	-	-
34	9104	DHIF	*11:01		-	-	-	-	-	-	-	-	-
35	9302	SSTO	*04:03		-	-	-	-	-	-	-	-	-
36	9024	KT17	*04:03	*04:06	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*13:01		-	-	-	-	-	-	-	-	-
38	9099	LZL	*14:02		-	-	-	-	-	-	-	-	-
39	9315	CML	*03:01	*04:01	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*07:01	*09:01	+	+	-	-	-	-	-	-	-
41	9055	H0301	*13:02		-	-	-	-	-	-	-	-	-
42	9066	TAB089	*08:03		-	-	-	-	-	-	-	-	-
43	9076	T7526	*09:01		+	+	-	-	-	-	-	-	-
44	9057	TEM	*14:01		-	-	-	-	-	-	-	-	-
45	9239	SHJO	*07:01		-	-	-	-	-	-	-	-	-
46	9013	SCHU	*15:01		-	-	-	-	-	-	-	-	-
47	9045	TUBO	*11:04	*12:01	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*01:03		-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS**Olerup SSP® DRB1*09 SSP****Product number:****101.120-06u – without *Taq* polymerase****Lot number:****60K****Expiry date:****2013-April-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	2010-781-06
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. In primer mix 6, one 3'-primer could not be tested.

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

Date of approval: 2010-November-09

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*09
Product number: 101.120-06u
Lot number: 60K

Intended use: DRB1*09 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 9001:2008 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-November-09

Olle Olerup
Managing Director

Lot No.: **60K**

Lot-specific Information

www.olerup-ssp.com

ADDRESSES:

Manufacturer:

Olerup SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

Tel: +46-8-717 88 27

Fax: +46-8-717 88 18

E-mail: info-ssp@olerup.com

Web page: <http://www.olerup-ssp.com>

Distributed by:

Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.

Tel: +43-1-710 15 00

Fax: +43-1-710 15 00 10

E-mail: support-at@olerup.com

Web page: <http://www.olerup.com>

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382

Tel: 1-877-OLERUP1

Fax: 610-344-7989

E-mail: info.us@olerup.com

Web page: <http://www.olerup.com>

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.