

101.120-06 – including *Taq* polymerase, IFU-01 Rev. No. 03101.120-06u – without *Taq* polymerase, IFU-02 Rev. No. 03Visit www.olerup-ssp.com for

“Instructions for Use” (IFU)

Lot No.: **40N**

Lot-specific information

Olerup SSP[®] DRB1*09

Product number:	101.120-06 – including <i>Taq</i> polymerase 101.120-06u – without <i>Taq</i> polymerase
Lot number:	40N
Expiry date:	2014-September-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. 40N.

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

The DRB1*09 kit is updated to enable separation of:

- Confirmed DRB1*09 alleles as listed in the IMGT/HLA database¹
- Polymorphisms in exons outside of the region encoding the peptide binding domain
- Null and Alternatively expressed alleles

The Lot-specific information for DRB1*09 including and without *Taq* polymerase is now described in one common Product Insert.

¹As described in section Uniquely Identified Alleles.

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. 60K**).

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

Well	5'-primer	3'-primer	rationale
4	-	Added	3'-primers added for the DRB1*09:13, 09:15, 09:16 and 09:17 alleles. 3'-primer from well 6 added for the DRB1*09:09 allele.
6	-	Added, moved	3'-primers added for the DRB1*09:10, 09:11 and 09:14 alleles. Primer for the DRB1*09:09 allele moved to well 4.
8	Added	-	5'-primer added for the DRB1*09:12 allele.

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PRODUCT DESCRIPTION**DRB1*09 SSP subtyping****CONTENT**

The primer set contains 5'- and 3'-primers for identifying the DRB1*09:01 to DRB1*09:17 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. '40N'.

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*09:17**, recognized by the HLA Nomenclature Committee in January 2012¹ will be amplified by the primers in the DRB1*09 subtyping kit.

The DRB1*09 kit enables separation of the confirmed DRB1*09 alleles as listed in the IMGT/HLA database. An HLA allele is listed as confirmed by IMGT/HLA if it has been sequenced by more than a single laboratory or from multiple sources. Current allele confirmation status for DRB1*09 alleles is listed below.

The DRB1*09 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles

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 2012-January-12, release 3.7.0, www.ebi.ac.uk/imgt/hla.

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ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹
DRB1*09:01:02	Confirmed	DRB1*09:07	Unconfirmed
DRB1*09:01:03	Unconfirmed	DRB1*09:08	Unconfirmed
DRB1*09:01:04	Unconfirmed	DRB1*09:09	Unconfirmed
DRB1*09:01:05	Unconfirmed	DRB1*09:10	Unconfirmed
DRB1*09:01:06	Confirmed	DRB1*09:11	Unconfirmed
DRB1*09:01:07	Unconfirmed	DRB1*09:12	Unconfirmed
DRB1*09:02:01	Unconfirmed	DRB1*09:13	Unconfirmed
DRB1*09:02:02	Unconfirmed	DRB1*09:14	Unconfirmed
DRB1*09:03	Unconfirmed	DRB1*09:15	Unconfirmed
DRB1*09:04	Confirmed	DRB1*09:16	Unconfirmed
DRB1*09:05	Unconfirmed	DRB1*09:17	Unconfirmed
DRB1*09:06	Confirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2012-January-12, release 3.7.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 23 alleles generate 10 amplification patterns that can be combined in 55 homozygous and heterozygous combinations. 43 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products were not considered in these calculations.

```

++++----- *09:02:01, *09:03 = *09:02:01, *09:16
+++--+-+-- *09:02:01, *09:05 = *09:02:01, *09:10
++-+++-+-- *09:03, *09:04 = *09:03, *09:08 = *09:04, *09:16 = *09:08, *09:16
+-+--+-+-- *09:03, *09:05 = *09:03, *09:10 = *09:10, *09:16
+-+--+-+-- *09:03, *09:06 = *09:06, *09:16
+-+--+-+-- *09:03, *09:07 = *09:07, *09:16
+-+--+-+-- *09:01:02, *09:03 = *09:01:02, *09:16 = *09:03, *09:03 = *09:03, *09:16
+-+--+-+-- *09:04, *09:05 = *09:04, *09:10 = *09:05, *09:08 = *09:08, *09:10
+-+--+-+-- *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, *09:10
+-+--+-+-- *09:05, *09:07 = *09:07, *09:10
+-+--+-+-- *09:01:02, *09:05 = *09:01:02, *09:10 = *09:05, *09:10 = *09:10, *09:10
+-+--+-+-- *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 = *09:02:01-09:02:02

*09:03 = *09:03, 09:09, 09:13, 09:15 and 09:17

*09:07 = *09:07 and 09:12

*09:10 = *09:10, 09:11 and 09:14

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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, 09:06-09:15, 09:17
2	130 bp	430 bp	*09:01:02-09:01:07, 09:03-09:07, 09:09-09:17
3	185 bp	430 bp	*09:02:01-09:02:02
4 ^{3,4}	100 bp, 130 bp, 215 bp, 245 bp, 275 bp	430 bp	*09:03, 09:09, 09:13, 09:15-09:17
5 ^{4,5}	185 bp, 215 bp	430 bp	*09:04, 09:08
6 ^{3,5,6}	105 bp, 150 bp, 250 bp	430 bp	*09:05, 09:10-09:11, 09:14
7	220 bp	430 bp	*09:06
8	175 bp	430 bp	*09:07, 09:12

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 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 125 base pairs have a lower intensity than longer PCR bands.

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⁴Primer mix 4: Specific PCR fragment of 100 bp in the DRB1*09:17 allele. Specific PCR fragment of 130 bp in the DRB1*09:13 allele. Specific PCR fragment of 215 bp in the DRB1*09:03 allele. Specific PCR fragment of 245 bp in the DRB1*09:15 and 09:16 alleles. Specific PCR fragment of 275 bp in the DRB1*09:09 allele.

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.

⁶Primer mix 6: Specific PCR fragment of 105 bp in the DRB1*09:10 allele. Specific PCR fragment of 150 bp in the DRB1*09:11 and 09:14 alleles. Specific PCR fragment of 250 bp in the DRB1*09:05 allele.

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INTERPRETATION TABLE								
DRB1*09 SSP subtyping								
Amplification patterns of the DRB1*09:01 to 09:17 alleles								
	Well ⁶							
	1	2	3	4	5	6	7	8
Length of spec.	195	130	185	100	185	105	220	175
PCR product				130	215	150		
				215		250		
				245				
				275				
Length of int.	515	430	430	430	430	430	430	430
pos. control ¹								
5'-primer ²	26(164) 5'-gTA 3'	28(170) 5'-gCA 3'	9(112) 5'-TgA 3'	9(112) 5'-TgA 3'	9(112) 5'-TgA 3'	9(112) 5'-TgA 3'	26(165) 5'-TAT 3'	12(123) 5'-AAC 3'
								13(125) 5'-gTA 3'
3'-primer(s) ³	78(319) 5'-CAC 3'	57(257) 5'-CgA 3'	57(257) 5'-CAT 3'	29(172) 5'-TCC 3'	57(256) 5'-gCT 3'	30(176) 5'-TgT 3'	86(344) 5'-CCA 3'	57(257) 5'-CgA 3'
			57(258) 5'-gCg 3'	39(203) 5'-AgA 3'	67(286) 5'-gAg 3'	44(218) 5'-CCC 3'		
				67(286) 5'-gAA 3'		48(229) 5'-CCC 3'		
				75(311) 5'-CCC 3'		78(319) 5'-gTA 3'		
				78(319) 5'-CAg 3'				
				87(348) 5'-CAT 3'				
Well No.	1	2	3	4	5	6	7	8
DRB1 allele ^{4,5}								
*09:01:02-09:01:07	1	2						
*09:02:01-09:02:02	1		3					
*09:03, 09:09, 09:13, 09:15, 09:17	1	2		4				
*09:04	1	2			5			
*09:05		2				6		
*09:06	1	2					7	
*09:07, 09:12	1	2						8
*09:08	1				5			
*09:10-09:11, 09:14	1	2				6		
*09:16		2		4				
DRB1 allele ⁴								
Well No.	1	2	3	4	5	6	7	8

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

²The codon, and in parenthesis the nucleotide, in the 2nd exon, 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 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*09011 allele has been shown to contain errors and be identical to DRB1*09:01:02.

⁵DRB1*09 alleles in bold lettering are listed as confirmed alleles on the IMGT/HLA web page www.ebi.ac.uk/imgt/hla, release 3.7.0, January 2012.

⁶Primer mix 4: Specific PCR fragment of 100 bp in the DRB1*09:17 allele. Specific PCR fragment of 130 bp in the DRB1*09:13 allele. Specific PCR fragment of 215 bp in the DRB1*09:03 allele. Specific PCR fragment of 245 bp in the DRB1*09:15 and 09:16 alleles. Specific PCR fragment of 275 bp in the DRB1*09:09 allele.

Primer 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 105 bp in the DRB1*09:10 allele. Specific PCR fragment of 150 bp in the DRB1*09:11 and 09:14 alleles. Specific PCR fragment of 250 bp in the DRB1*09:05 allele.

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CELL LINE VALIDATION SHEET												
DRB1*09 SSP subtyping kit												
				Production No.	Well							
					1	2	3	4	5	6	7	8
					201297901	201297902	200961603	201297904	200961605	201297906	201297907	201297908
	IHWC 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		-	-	-	-	-	-	-	-

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CERTIFICATE OF ANALYSIS**Olerup SSP® DRB1*09 SSP**

Product number:

101.120-06 – including *Taq* polymerase**101.120-06u – without *Taq* polymerase**

Lot number:

40N

Expiry date:

2014-September-01

Number of tests:

6

Number of wells per test:

8**Well specifications:**

Well No.	Production No.
1	2012-979-01
2	2012-979-02
3	2009-616-03
4	2012-979-04
5	2009-616-05
6	2012-979-06
7	2012-979-07
8	2012-979-08

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 8, one 5'-primer could not be tested, and in primer mixes 4 and 6 five respectively three 3'-primers could not be tested.

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

Date of approval: 2012-March-09

Approved by:

Production Quality Control

101.120-06 – including *Taq* polymerase, IFU-01 Rev. No. 03
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Declaration of Conformity

Product name: *Olerup* SSP® DRB1*09

Product number: 101.120-06/06u

Lot number: 40N

Intended use: DRB1*09 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Franzengatan 5
SE-112 51 Stockholm, 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, Franzengatan 5, SE-112 51 Stockholm, Sweden.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom. (Notified Body number: 0088.)

Stockholm, Sweden
2012-March-09

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

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Lot-specific information

ADDRESSES:

Manufacturer:

Olerup SSP AB, Franzengatan 5, SE-112 51 Stockholm, 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**.