101.120-06 – including *Taq* polymerase, IFU-01
101. 120-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for **"Instructions for Use" (IFU)**

Lot No.: 14S

Lot-specific information

Olerup SSP® DRB1*09

Product number:	**101.120-06 – including *Taq* polymerase** **101.120-06u – without *Taq* polymerase**
Lot number:	**14S**
Expiry date:	**2015-November-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. 14S.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® DRB1*09 LOT (40N)

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

- Confirmed DRB1*09 alleles as listed in the IMGT/HLA database[1]
- 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.

[1]As 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. 40N)**.

Lot No.: 14S **Lot-specific information**

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

Well	5'-primer	3'-primer	rationale
2	-	Added	3'-primers added for the DRB1*09:01:08 and DRB1*09:01:09 alleles.
3	Exchanged	Exchanged	Primer pair exchanged for the DRB1*09:02 allele.
4	-	Added	3'-primer added for the DRB1*09:18 allele.
8	Exchanged, added	-	5'-primer exchanged for resolution of the DRB1*09:20 allele, 5'-primer added for the DRB1*09:19 allele

101.120-06 – including *Taq* polymerase, IFU-01
101. 120-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for **"Instructions for Use" (IFU)**

Lot No.: 14S **Lot-specific information**

PRODUCT DESCRIPTION

DRB1*09 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*09:01 to DRB1*09:20 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. '14S'.
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*09:20**, recognized by the HLA Nomenclature Committee in January 2013[1] 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 silent mutations DRB1*09:01:02 to DRB1*09:01:09 or the DRB1*09:02:01 and DRB1*09:02:02 alleles.

[1]DRB alleles listed on the IMGT/HLA web page 2013-January-11, release 3.11.0, www.ebi.ac.uk/imgt/hla.

101.120-06 – including *Taq* polymerase, IFU-01
101. 120-06u – without *Taq* polymerase, IFU-02

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Lot No.: 14S **Lot-specific information**

ALLELE CONFIRMATION STATUS

Allele	Status[1]	Allele	Status[1]
DRB1*09:01:02	**Confirmed**	DRB1*09:09	Unconfirmed
DRB1*09:01:03	Unconfirmed	DRB1*09:10	Unconfirmed
DRB1*09:01:04	Unconfirmed	DRB1*09:11	Unconfirmed
DRB1*09:01:05	Unconfirmed	DRB1*09:12	Unconfirmed
DRB1*09:01:06	**Confirmed**	DRB1*09:13	Unconfirmed
DRB1*09:01:07	Unconfirmed	DRB1*09:14	Unconfirmed
DRB1*09:01:08	Unconfirmed	DRB1*09:15	Unconfirmed
DRB1*09:01:09	Unconfirmed	DRB1*09:16	Unconfirmed
DRB1*09:02:01	Unconfirmed	DRB1*09:17	Unconfirmed
DRB1*09:02:02	Unconfirmed	DRB1*09:18	Unconfirmed
DRB1*09:03	Unconfirmed	DRB1*09:19	Unconfirmed
DRB1*09:04	**Confirmed**	DRB1*09:20	Unconfirmed
DRB1*09:05	Unconfirmed		
DRB1*09:06	**Confirmed**		
DRB1*09:07	Unconfirmed		
DRB1*09:08	Unconfirmed		

[1]Allele status "confirmed" or "unconfirmed" as listed on the IMGT/HLA web page 2013-January-11, release 3.11.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 28 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.

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

*09:01:02 = *09:01:02-09:01:09
*09:02:01 =*09:02:01-09:02:02
*09:03 = *09:03, 09:09, 09:13, 09:15 and 09:17-09:18
*09:07 = *09:07, 09:12 and 09:19-09:20
*09:10 = *09:10, 09:11 and 09:14

101.120-06 – including *Taq* polymerase, IFU-01
101. 120-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for **"Instructions for Use" (IFU)**

Lot No.: 14S

Lot-specific information

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, 09:17-09:20
2[4]	130 bp	430 bp	*09:01:02-09:01:09, 09:03-09:07, 09:09-09:20
3[4]	135 bp	430 bp	*09:02:01-09:02:02
4[3,5,6]	100 bp, 130 bp, 215 bp, 245 bp, 275 bp	430 bp	*09:03, 09:09, 09:13, 09:15-09:18
5[7]	185 bp, 215 bp	430 bp	*09:04, 09:08
6[3,8]	105 bp, 150 bp, 250 bp	430 bp	*09:05, 09:10-09:11, 09:14
7	220 bp	430 bp	*09:06
8[9]	150 bp, 180 bp	430 bp	*09:07, 09:12, 09:19-09:20

[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 HLA-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits the respective lengths of the HLA-specific PCR product(s) are given for the alleles amplified by these primer mixes.
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]HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.
[4]Primer mixes 2 and 3 may have tendencies of unspecific amplifications.

101.120-06 – including *Taq* polymerase, IFU-01
101. 120-06u – without *Taq* polymerase, IFU-02

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Lot No.: 14S **Lot-specific information**

[5]Primer mix 4 has a tendency to giving rise to primer oligomer formation.
[6]Primer 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 and 09:18 alleles. 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.
[7]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.
[8]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.
[9]Primer mix 8: Specific PCR fragment of 150 bp in the DRB1*09:19 allele. Specific PCR fragment of 180 bp in the DRB1 *09:07, 09:12 and 09:20 alleles.

101.120-06 – including *Taq* polymerase, IFU-01
101. 120-06u – without *Taq* polymerase, IFU-02

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Lot No.: 14S **Lot-specific information**

INTERPRETATION TABLE

DRB1*09 SSP subtyping

Amplification patterns of the DRB1*09:01 to 09:20 alleles

	Well[6]							
	1	2	3	4	5	6	7	8
Length of spec. PCR product	195	130	135	100 130 215 245 275	185 215	105 150 250	220	150 180
Length of int. pos. control[1]	515	430	430	430	430	430	430	430
5'-primer[2]	26(164) 5' -gTA 3'	28(170) 5' -gCA 3'	28(170) 5' -gCA 3'	8(112) 5' -TgA 3'	8(112) 5' -TgA 3'	8(112) 5' -TgA 3'	26(165) 5' -TAT 3'	8(112) 5' -TgC 3' 12(123) 5' -AAC 3' 21(149) 5' -gAT 3'
3'-primer(s)[3]	77(319) 5' -CAC 3'	57(257) 5' -CgA 3' 58(261) 5' -TCC 3' 58(261) 5' -TCA 3'	60(266) 5' -AgT 3'	28(172) 5' -TCC 3' 39(203) 5' -AgA 3' 63(276) 5' -TgT 3' 66(286) 5' -gAA 3' 75(311) 5' -CCC 3' 77(319) 5' -CAg 3' 87(348) 5' -CAT 3'	56(256) 5' -gCT 3' 66(286) 5' -gAg 3'	30(176) 5' -TgT 3' 44(218) 5' -CCC 3' 47(229) 5' -CCC 3' 77(319) 5' -gTA 3'	86(344) 5' -CCA 3'	57(257) 5' -CgA 3'
Well No.	1	2	3	4	5	6	7	8
DRB1 allele[4,5]								
***09:01:02**-09:01:09	1	2						
*09:02:01-09:02:02	1		3					
*09:03, 09:09, 09:13, 09:15, 09:17-09:18	1	2		4				
***09:04**	1	2			5			
*09:05		2				6		
***09:06**	1	2					7	
*09:07, 09:12, 09:19-09:20	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

CE 0088

101.120-06 – including *Taq* polymerase, IFU-01
101. 120-06u – without *Taq* polymerase, IFU-02

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Lot No.: 14S **Lot-specific information**

[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, 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.
[3]The 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.
[4]The nucleotide sequence of the DRB1*09011 allele has been shown to contain errors and be identical to DRB1*09:01:02.
[5]DRB1*09 alleles in bold lettering are listed as confirmed alleles on the IMGT/HLA web page www.ebi.ac.uk/imgt/hla, release 3.11.0, January 2013.
[6]Primer 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 and 09:18 alleles. 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.
Primer mix 8: Specific PCR fragment of 150 bp in the DRB1*09:19 allele. Specific PCR fragment of 180 bp in the DRB1 *09:07, 09:12 and 09:20 alleles.

101.120-06 – including *Taq* polymerase, IFU-01
101. 120-06u – without *Taq* polymerase, IFU-02

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Lot No.: 14S **Lot-specific information**

CELL LINE VALIDATION SHEET

DRB1*09 SSP subtyping kit

					Well							
					1	2	3	4	5	6	7	8
				Production No.	201297901	201316402	201316403	201316404	200961605	201297906	201297907	201316408
	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		-	-	-	-	-	-	-	-

Lot No.: 14S **Lot-specific information**

CERTIFICATE OF ANALYSIS

Olerup SSP® DRB1*09 SSP

Product number: **101.120-06 – including *Taq* polymerase**
101.120-06u – without *Taq* polymerase

Lot number: **14S**
Expiry date: **2015-November-01**
Number of tests: **6**
Number of wells per test: **8**

Well specifications:

Well No.	Production No.
1	2012-979-01
2	2013-164-02
3	2013-164-03
4	2013-164-04
5	2009-616-05
6	2012-979-06
7	2012-979-07
8	2013-164-08

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, two 5'-primers could not be tested, and in primer mixes 4 and 6 six respectively three 3'-primers could not be tested. Additional primers in primer solutions 2 were tested by separately adding an additional 5'-primer.

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

Date of approval: 2013-May-30

Approved by:

Production Quality Control

101.120-06 – including *Taq* polymerase, IFU-01
101. 120-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
"Instructions for Use" (IFU)

Lot No.: 14S **Lot-specific information**

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*09
Product number: 101.120-06/06u
Lot number: 14S

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 13485:2012, 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
2013-May-30

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

101.120-06 – including *Taq* polymerase, IFU-01
101. 120-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for **"Instructions for Use" (IFU)**

Lot No.: 14S **Lot-specific information**

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