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

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"Instructions for Use" (IFU)

Lot No.: 4G1

Lot-specific information

Olerup SSP® DRB1*09

Product number:	**101.120-06 – including *Taq* polymerase** **101.120-06u – without *Taq* polymerase**
Lot number:	**4G1**
Expiry date:	**2021-01-01**
Number of tests:	**6**
Number of wells per test:	**15+1**
Storage - pre-aliquoted primers:	**dark at -20ºC**
- PCR Master Mix:	**-20ºC**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 4G1.

Complete product documentation consists of generic Instructions for Use (IFU), lot specific Product Insert, Worksheet and Certificate.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® DRB1*09 LOT (5F9)

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

One well has been added to DRB1*09, well **16**.

The format of the Worksheet has been changed.

[1]As described in section Uniquely Identified Alleles.

The DRB1*09 primer set, specificity and interpretation tables have been updated for the DRB alleles described since the previous *Olerup* SSP® DRB1*09 lot was made **(Lot No. 5F9)**. The kit design is based on IMGT/HLA databased 3.32.0.

Lot No.: 4G1 **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
15	Added	Added	Negative Control moved to well 16, primer pair added for the DRB1*09:31 allele.
16	-	-	Negative Control added from well 15.

Lot No.: 4G1 **Lot-specific information**

Well **16** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup* SSP® HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

HLA-specific PCR product sizes range from 75 to 200 base pairs.
The PCR product generated by the positive control primer pair is 430 base pairs.

Length of PCR product	**105**	**200**	**105**	**80**	**75**	**80**	**85**
5'-primer[1]	**164**	**340**	**440**	**45**	**45**	**43**	**36**
	5'-CAC3'	**5'-Agg3'**	**5'-TTA3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-TAC3'**
							36
							5'-TAT3'
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**	**47**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**	**5'-ACA3'**
							48
							5'-gCA3'
							48
							5'-gCC3'
							52
							5'-TgT3'
A*	**+**	**+**	**+**				
B*	**+**	**+**	**+**				
C*	**+**	**+**	**+**				
DRB1				**+**	**+**		
DRB3				**+**	**+**		
DRB5				**+**			
DQB1					**+**		
DPB1						**+**	
DQA1							**+**

[1]The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and codonnumbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

[2]The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and 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.

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

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Lot No.: 4G1 **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*09:32 alleles.

PLATE LAYOUT

Each test consists of 16 PCR reactions in a 16 well PCR plate.

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
9	**10**	**11**	**12**	**13**	**14**	**15**	**NC**

The 16 well PCR plate is marked with 'DRB1*09' in silver/gray ink.
Well No. 1 is marked with the Lot No. '4G1'.
Wells 1 to 15 – DRB1*09 high resolution primers.
Well 16 – Negative Control (NC).
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 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

Due to the sharing of sequence motifs between DRB1 alleles, non-DRB1*09 alleles will be amplified by some primer mixes. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the DRB1*09 alleles, i.e. **DRB1*09:01 to DRB1*09:32**, recognized by the HLA Nomenclature Committee in April 2018[1,2] 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.

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

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Lot No.: 4G1 **Lot-specific information**

The following DRB1*09 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix
DRB1*09:09, 09:13, 09:17-09:18	14

[1]DRB alleles listed on the IMGT/HLA web page 2018-April-16, release 3.32.0, www.ebi.ac.uk/imgt/hla.
[2]Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page http://hla.alleles.org/alleles/deleted.html.

ALLELE CONFIRMATION STATUS

Allele	Status[1]	Allele	Status[1]	Allele	Status[1]
DRB1*09:01:02:01	**Confirmed**	DRB1*09:10	Unconfirmed	DRB1*09:30	Unconfirmed
DRB1*09:01:02:02	Unconfirmed	DRB1*09:11	Unconfirmed	DRB1*09:31	Unconfirmed
DRB1*09:01:03	Unconfirmed	DRB1*09:12	Unconfirmed	DRB1*09:32	Unconfirmed
DRB1*09:01:04	Unconfirmed	DRB1*09:13	Unconfirmed		
DRB1*09:01:05	Unconfirmed	DRB1*09:14	Unconfirmed		
DRB1*09:01:06	**Confirmed**	DRB1*09:15	Unconfirmed		
DRB1*09:01:07	Unconfirmed	DRB1*09:16	Unconfirmed		
DRB1*09:01:08	Unconfirmed	DRB1*09:17	Unconfirmed		
DRB1*09:01:09	Unconfirmed	DRB1*09:18	Unconfirmed		
DRB1*09:01:10	**Confirmed**	DRB1*09:19	Unconfirmed		
DRB1*09:01:11	Unconfirmed	DRB1*09:20	Unconfirmed		
DRB1*09:02:01	Unconfirmed	DRB1*09:21	Unconfirmed		
DRB1*09:02:02	Unconfirmed	DRB1*09:22	Unconfirmed		
DRB1*09:03	Unconfirmed	**DRB1*09:23**	**Confirmed**		
DRB1*09:04	**Confirmed**	DRB1*09:24	Unconfirmed		
DRB1*09:05	Unconfirmed	**DRB1*09:25**	**Confirmed**		
DRB1*09:06	**Confirmed**	DRB1*09:26	Unconfirmed		
DRB1*09:07	Unconfirmed	**DRB1*09:27**	**Confirmed**		
DRB1*09:08	Unconfirmed	DRB1*09:28	Unconfirmed		
DRB1*09:09	Unconfirmed	DRB1*09:29	Unconfirmed		

[1]Allele status "confirmed" or "unconfirmed" as listed on the IMGT/HLA web page 2018-April-16, release 3.32.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DRB1*09 homo- and heterozygotes is available upon request.

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

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Lot No.: 4G1

Lot-specific information

SPECIFICITY TABLE

DRB1*09 SSP subtyping

Specificities and sizes of the PCR products of the 15+1 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[3]	Other amplified DRB1 alleles
1	250 bp	**515 bp**	*09:01:02:01-09:04, 09:06, 09:08-09:15, 09:17-09:19, 09:21-09:32	
2	135 bp	430 bp	*09:01:02:01-09:01:11, 09:03-09:07, 09:09-09:22, 09:25-09:31	
3	140 bp	430 bp	*09:02:01-09:02:02	
4	215 bp 245 bp	430 bp	*09:03 *09:15, 09:16	
5	185 bp 215 bp	430 bp	*09:08 *09:04	 *01:65:01
6[4]	105 bp 150 bp 250 bp	430 bp	*09:10 *09:11, 09:14 *09:05	 *01:65:01
7	220 bp	430 bp	*09:06	
8	150 bp 180 bp	430 bp	*09:19 *09:07, 09:12, 09:20	*07:52
9	130 bp	**515 bp**	*09:22	
10[4,5]	75 bp	**515 bp**	*09:21	
11	195 bp	430 bp	*09:23	*04:90, 04:155, 04:167, 07:50, 13:220, 16:42, **DRB5*01:12, DRB5*01:15**
12	235 bp	430 bp	*09:25	
13[4]	80 bp	430 bp	*09:27	
14[4]	100 bp 130 bp 205 bp 275 bp	430 bp	*09:17 *09:13 *09:18 *09:09	
15[4]	95 bp	430 bp	*09:31	
16[6]	-	-	**Negative Control**	

[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 alleles listed are specified according to amplicon length.
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.

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

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Lot No.: 4G1 **Lot-specific information**

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 internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the longer, 515 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

[3]For several DRB1 alleles 1st and/or 3rd exon(s) and beyond, as well as intron nucleotide sequences, are not available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

[4]HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

[5]Primer mix 10 may have tendencies of unspecific amplifications.

[6]Primer mix 16 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by the control primer pairs matching the human growth hormone gene. HLA-specific PCR product sizes range from 75 to 200 base pairs and the PCR product generated by the HGH positive control primer pair is 430 base pairs.

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

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Lot No.: 4G1

Lot-specific information

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	250	135	140	215	185	105	220	150	130	75	195	235
PCR product				245	215	150		180				
						250						
Length of int.	515	430	430	430	430	430	430	430	515	515	430	430
pos. control[1]												
5'-primer(s)[2]	9(112)	26(165)	26(165)	9(112)	9(112)	9(112)	26(165)	9(112)	9(112)	196(674)	26(165)	26(165)
	5' -TgA 3'	5' -TAT 3'	5' -TAT 3'	5' -TgA 3'	5' -TgA 3'	5' -TgA 3'	5' -TAT 3'	5' -TgC 3'	5' -TgA 3'	5' -ACg 3'	5' -TTC 3'	5' -TAT 3'
								12(123)				
								5' -AAC 3'				
								21(149)				
								5' -gAT 3'				
3'-primer(s)[3]	78(319)	57(257)	60(266)	67(286)	57(256)	30(176)	86(344)	57(257)	39(203)	207(706)	78(319)	91(359)
	5' -CAC 3'	5' -CgA 3'	5' -AgT 3'	5' -gAA 3'	5' -gCT 3'	5' -TgT 3'	5' -CCA 3'	5' -CgA 3'	5' -AgT 3'	5' -CAC 3'	5' -CAC 3'	5' -gCg 3'
		58(261)		75(311)	67(286)	44(218)						
		5' -TCA 3'		5' -CCC 3'	5' -gAg 3'	5' -CCC 3'						
		58(261)		78(319)		48(229)						
		5' -TCC 3'		5' -CAg 3'		5' -CCC 3'						
						78(319)						
						5' -gTA 3'						
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15
Length of spec.	80	100	95
PCR product		130	
		205	
		275	
Length of int.	430	430	430
pos. control[1]			
5'-primer(s)[2]	26(165)	9(112)	-22(25)
	5' -TAT 3'	5' -TgA 3'	5' -gAT 3'
3'-primer(s)[3]	39(202)	29(172)	-4(79)
	5' -gCT 3'	5' -TCC 3'	5' -AgC 3'
		39(203)	
		5' -AgA 3'	
		63(276)	
		5' -TgT 3'	
		87(348)	
		5' -CAT 3'	
Well No.	13	14	15

[1]The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the longer, 515 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

[2]The nucleotide position matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

[3]The nucleotide position matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

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

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Lot No.: 4G1 **Lot-specific information**

CELL LINE VALIDATION SHEET

DRB1*09 SSP subtyping kit[2]

					Well														
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
				Production No.	201559601	201559602	201559603	201784004	201559605	201559606	201559607	201559608	201559609	201559610	201559611	201784012	201784013	201784014	201892315
	IHWC cell line[1]		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		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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

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Lot No.: 4G1 **Lot-specific information**

[1]The provided cell line HLA specificities are retrieved from the http://www.ihwg.org/hla web site. The specificity of an individual cell line may thus be subject to change.

[2]The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs.

No DNAs carrying the allele to be amplified by primer solutions 3 to 15 were available. The specificities of the primers in primer solutions 3 to 8 and 11 were tested by separately adding one or two additional 5'-primers, respectively one additional 3'-primer.

In primer solutions 10 and 15 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solutions 9 and 12 to 14 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer mix 8, two 5'-primers could not be tested, and in primer mixes 4 and 6 two respectively three 3'-primers could not be tested. Additional primers in primer solutions 2 were tested by separately adding additional 5'-primers.

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

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

Lot No.: 4G1 **Lot-specific information**

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