Product Insert

Visit https://labproducts.caredx.com for

"Instructions for Use" (IFU)

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

Lot No.: **0M8**

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:	0M8
Expiry date:	2025-03-01
Number of tests:	6
Number of wells per test:	15+1
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. 0M8.

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 (5K5)

- The product documentation has been updated for new alleles of IMGT 3.43.0
- The kit resolution focuses on common and well documented (CWD) alleles¹.

¹As 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 5K5).

Well	5'-primer	3'-primer	rationale
11	Added	-	5'-primer added for improved resolution of
			the DRB1*09:07 and 09:20 alleles.

¹S. J. Mack, P. Cano, J. A. Hollenbach et al. Common and well-documented HLA alleles: 2012 update to the CWD catalogue. Tissue Antigens, 2013, 81, 194–203



Product Insert

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Well **16** contains <u>Negative Control primer pairs</u>, that will amplify the majority 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.

Lot-specific information

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

Length of PCR	105	200	105	80	75	80	85
product							
5'-primer ¹	164	340	440	45	45	43	36
•	^{5'} -CAC ^{3'}	^{5'} -Agg ^{3'}	^{5'} -TTA3'	^{5'} -Tgg ^{3'}	^{5′} -Tgg³′	^{5′} -Tgg³′	^{5'} -TAC ^{3'}
							36
							^{5'} -TAT ^{3'}
3'-primer ²	231	2 nd I	507	59	58	57	47
	^{5'} -TgC ^{3'}	⁵ '-AAA ^{3'}	⁵ '-TTg ^{3'}	^{5'} -CTC ^{3'}	^{5'} -ggC ^{3'}	^{5'} -CTC ^{3'}	^{5'} -ACA ^{3'}
							48
							^{5'} -gCA ^{3'}
							48
							^{5'} -gCC ^{3'}
							52
							^{5′} -TgT³′
A*	+	+	+				
B*	+	+	+				
C*	+	÷	÷				
DRB1				÷	+		
DRB3				+	+		
DRB5				+			
DQB1					+		
DPB1						+	
DQA1							+

¹The 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 <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given.

²The 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 specificitydetermining 3'-end of the primer is given in the anti-sense direction. Nucleotide and codon numbering as on the <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given. **Product Insert**

101.120-06 – including *Taq* polymerase, IFU-01 101.120-06u – without *Taq* polymerase, IFU-02 Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

Lot No.: **0M8**

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*09:45 alleles.

PLATE LAYOUT

Each test consists of 16 PCR reactions in a 16 well PCR plate. **Note:** This lot was manufactured using white plastic trays.

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. '0M8'.

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*09:45**, recognized by the HLA Nomenclature Committee in January 2021^{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 3.32.0. 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 many null and alternatively expressed alleles.

Product Insert

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Lot No.: **0M8**

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

¹DRB alleles listed on the IMGT/HLA web page 2021-January-18, release 3.43.0, <u>www.ebi.ac.uk/imgt/hla</u>.

²Alleles 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 <u>http://hla.alleles.org/alleles/deleted.html</u>.

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		

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

RESOLUTION IN HOMO- AND HETEROZYGOTES

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



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Lot No.: **OM8**

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, 09:21:01:01-09:45	
2	135 bp	430 bp	*09:01:02:01-09:01:11, 09:03-09:07, 09:09-09:22, 09:25-09:31, 09:33-09:41, 09:43, 09:45	
3	140 bp	430 bp	*09:02:01-09:02:02, 09:42	
4	215 bp 245 bp	430 bp	*09:03 *09:15-09:16	
5	185 bp 215 bp	430 bp	*09:08 *09:04, 09:42	*01:65:01, DRB4*01:125
6 ⁴	105 bp 150 bp 250 bp	430 bp	*09:10 *09:11, 09:14 *09:05	*01:65:01, DRB4*01:125
7	220 bp	430 bp	*09:06	01.03.01, DKD4 01.123
8	150 bp 180 bp	430 bp	*09:19 *09:07, 09:12, 09:20	*07:52
9	130 bp	515 bp	*09:22	
9 10 ⁴	75 bp	515 bp	*09:21:01:01-09:21:01:02	DRB4*01:124
11	195 bp 235 bp	430 bp	*09:23	*04:90, 04:155, 04:167, 07:50, 13:220, 16:42, DRB5*01:12, DRB5*01:15 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 ⁵	-	-	Negative Control	

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 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.

Product Insert

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

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Lot No.: **OM8**

Lot-specific information

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.

²The 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.

³For 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.

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

⁵Primer mix 16 contains a negative control, which will amplify the majority 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 200 base pairs.

Product Insert

101.120-06 – including *Taq* polymerase, IFU-01 101.120-06u – without *Taq* polymerase, IFU-02 Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

Lot No.: **0M8**

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			235	
						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)	13(125)	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'} -gTA ^{3'}	^{5'} -TAT ^{3'}
								12(123)			26(165)	
								5' -AAC 3'			5' -TTC 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' -CAC 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

¹The 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.

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

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



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

Lot No.: **0M8**

Lot-specific information

	CELL LINE VALIDATION SHEET																		
			DRB	1*09 S	<u>SP</u>	su	bty	/pi	ng	kit	2								
												We							
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
				tion	60	902	903	904	905	906	907	908	606	910	911	912	913	914	915
				quet	129	129	129	129	129	129	129	129	129	129	129	129	129	129	129
				Production No.	202129901	202129902	202129903	202129904	202129905	202129906	202129907	202129908	202129909	202129910	202129911	202129912	202129913	202129914	202129915
	11.1\A	/C cell line ¹		 RB1															
1	9001	-	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2		LK707	*15:02	*04:05	-	-	-	-	-	-	-	-	-	-	-		-	-	-
3		E4181324	*15:02	04.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4		GU373	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5		KAS011	*16:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*04:07	*08:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025	DEU	*04:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9		YAR	*04:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10		LKT3	*04:05		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11		PITOUT	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052		*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13		JESTHOM	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14		OLGA	*08:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15 16		DKB SWEIG007	*09:01		+	+	-	-	-	-	-	-		-	-	-	-	-	-
17		CTM3953540	*03:01	*13:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18		32367	*09:01	*11:01	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
19		BM16	*12:01	11.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20		SLE005	*13:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21		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		WT49	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27		CH1007	*04:05	*10:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28		BEL5GB	*04:16	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29		MOU	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30 31		RSH DUCAF	*03:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31		HAG	*03:01 *13:03	_	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33		MT14B	*04:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34		DHIF	*11:01	_	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35		SSTO	*04:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36		KT17	*04:03	*04:06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37		ННКВ	*13:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*14:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*03:01	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*07:01	*09:01	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
41		H0301	*13:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42		TAB089	*08:03	_	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43		T7526	*09:01		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
44		TEM	*14:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45		SHJO	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46		SCHU	*15:01	*12.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47			*11:04	*12:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*01:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Product Insert

101.120-06 – including *Taq* polymerase, IFU-01 101.120-06u – without *Taq* polymerase, IFU-02 Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

Lot No.: 0M8

Lot-specific information

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

²The 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, and one additional 3'-primer accordingly.

In primer solutions 10 and 15 it was only possible to test the 3'-primer, the 5'-primer was not possible to be tested. In primer solutions 9 and 12 to 14 it was only possible to test the 5'-primers, the 3'-primers were not possible to be tested.

In primer mix 8, two 5'-primers were not possible to be tested, and in primer mixes 4 and 6 one or more 3'primers could not be tested.

. In addition, two 3'-primers in primer solution 2 were tested by separately adding one or more 5'-primers.

Product Insert

101.120-06 – including *Taq* polymerase, IFU-01 101.120-06u – without *Taq* polymerase, IFU-02 Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

Lot No.: 0M8 ADDRESSES:

Lot-specific information

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