

101.126-12 – including *Taq* polymerase, IFU-01
 101.126-12u – without *Taq* polymerase, IFU-02

Visit <https://labproducts.caredx.com> for
 “Instructions for Use” (IFU)

Lot No.: **7L3**

Lot-specific information
Olerup SSP® DRB1*16

Product number:	101.126-12 – including <i>Taq</i> polymerase 101.126-12u – without <i>Taq</i> polymerase
Lot number:	7L3
Expiry date:	2024-11-01
Number of tests:	12
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. 7L3.

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*16 Lot (2L0)**

- The product documentation has been updated for new alleles of IMGT 3.41.0.
- The kit resolution focuses on common and well documented (CWD) alleles¹.

¹As described in section Uniquely Identified Alleles.

The DRB1*16 primer set, specificity and interpretation tables have been updated for the DRB1 alleles described since the previous *Olerup SSP®* DRB1*16 lot was made (**Lot No. 2L0**).

The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot (**Lot No. 2L0**).

Well	5'-primer	3'-primer	rationale
12	Added	-	5'-primer added for the DRB1*16:22 allele.
14	Added	-	5'-primer added for the DRB1*16:22 allele.

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

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Well **16** contains Negative Control primer pairs, 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.

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 product	105	200	105	80	75	80	85
5'-primer¹	164	340	440	45	45	43	36
	5'-CAC ^{3'}	5'-Agg ^{3'}	5'-TTA ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-TAC ^{3'}
							36
							5'-TAT ^{3'}
3'-primer²	231	2nd 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'-TgT ^{3'}
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 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.

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 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.

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PRODUCT DESCRIPTION

DRB1*16 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*16:01 to DRB1*16:65 alleles.

PLATE LAYOUT

Each test consists of 16 PCR reactions in a 16 well cut 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 cut PCR plate is marked with 'DRB1*16' in silver/gray ink.

Well No. 1 is marked with the Lot No. '7L3'.

Wells 1 to 15 – DRB1*16 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*16 alleles will be amplified by some primer mixes. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the DRB1*16 alleles, i.e. **DRB1*16:01 to DRB1*16:65**, recognized by the HLA Nomenclature Committee in July 2020^{1,2} will be amplified by the primers in the DRB1*16 subtyping kit.

The DRB1*16 kit enables separation of the confirmed DRB1*16 alleles as listed in the IMGT/HLA database 3.33.0. 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*16 alleles is listed below.

The DRB1*16 kit also enables identification of many null and alternatively expressed alleles.

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The following DRB1*16 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix
DRB1*16:03, 16:30	4

¹DRB1 alleles listed on the IMGT/HLA web page 2020-July-13, release 3.41.0, www.ebi.ac.uk/imgt/hla.

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 <http://hla.alleles.org/alleles/deleted.html>.

ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
DRB1*16:01:01	Confirmed	DRB1*16:09:01	Confirmed	DRB1*16:37	Confirmed
DRB1*16:01:02	Unconfirmed	DRB1*16:09:02	Unconfirmed	DRB1*16:38:01	Unconfirmed
DRB1*16:01:03	Unconfirmed	DRB1*16:10:01	Confirmed	DRB1*16:38:02	Confirmed
DRB1*16:01:04	Unconfirmed	DRB1*16:10:02	Confirmed	DRB1*16:39	Unconfirmed
DRB1*16:01:05	Confirmed	DRB1*16:11	Unconfirmed	DRB1*16:40	Unconfirmed
DRB1*16:01:06	Unconfirmed	DRB1*16:12	Confirmed	DRB1*16:41N	Confirmed
DRB1*16:01:07	Confirmed	DRB1*16:13N	Unconfirmed	DRB1*16:42	Unconfirmed
DRB1*16:01:08	Confirmed	DRB1*16:14	Confirmed	DRB1*16:43	Unconfirmed
DRB1*16:01:09	Unconfirmed	DRB1*16:15	Confirmed	DRB1*16:44	Unconfirmed
DRB1*16:01:10	Confirmed	DRB1*16:16	Unconfirmed	DRB1*16:45	Unconfirmed
DRB1*16:01:11	Unconfirmed	DRB1*16:17	Unconfirmed	DRB1*16:46	Confirmed
DRB1*16:01:12	Unconfirmed	DRB1*16:18	Unconfirmed	DRB1*16:47	Unconfirmed
DRB1*16:01:13	Unconfirmed	DRB1*16:19	Unconfirmed	DRB1*16:48	Unconfirmed
DRB1*16:01:14	Unconfirmed	DRB1*16:20	Unconfirmed		
DRB1*16:02:01:01	Confirmed	DRB1*16:21N	Unconfirmed		
DRB1*16:02:01:02	Unconfirmed	DRB1*16:22	Unconfirmed		
DRB1*16:02:01:03	Unconfirmed	DRB1*16:23	Unconfirmed		
DRB1*16:02:02	Unconfirmed	DRB1*16:24	Unconfirmed		
DRB1*16:02:03	Unconfirmed	DRB1*16:25	Confirmed		
DRB1*16:02:04	Unconfirmed	DRB1*16:26	Unconfirmed		
DRB1*16:02:05	Unconfirmed	DRB1*16:27	Unconfirmed		
DRB1*16:02:06	Confirmed	DRB1*16:28	Unconfirmed		
DRB1*16:02:07	Unconfirmed	DRB1*16:29	Unconfirmed		
DRB1*16:03	Unconfirmed	DRB1*16:30	Confirmed		
DRB1*16:04:01	Confirmed	DRB1*16:31	Unconfirmed		
DRB1*16:04:02	Confirmed	DRB1*16:32	Unconfirmed		
DRB1*16:05:01	Confirmed	DRB1*16:33	Unconfirmed		
DRB1*16:05:02	Confirmed	DRB1*16:34	Unconfirmed		
DRB1*16:07	Confirmed	DRB1*16:35	Confirmed		
DRB1*16:08	Confirmed	DRB1*16:36	Unconfirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2018-July-11, release 3.33.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DRB1*16 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

DRB1*16 SSP subtyping

Specificities and sizes of the PCR products of the 15+1 primer mixes used for DRB1*16 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB1*16 alleles ³	Other amplified DRB1 alleles
1	260 bp	515 bp	*16:01:01-16:05:02, 16:07-16:11, 16:13N-16:14, 16:16-16:22, 16:24- 16:32, 16:35, 16:37, 16:39-16:52, 16:54-16:57, 16:59Q-16:65	*15:02:01:01-15:02:19, 15:08, 15:11:01-15:11:02, 15:14-15:15:03, 15:19, 15:26-15:27, 15:29- 15:31:02, 15:34, 15:38-15:39, 15:44, 15:47, 15:58, 15:60, 15:63, 15:68, 15:78, 15:80N, 15:99, 15:101, 15:103-15:105:02, 15:115N, 15:118-15:119, 15:122, 15:126, 15:131, 15:140, 15:149, 15:152, 15:154N-15:156, 15:159N, 15:161, 15:167, 15:179
2	200 bp	515 bp	*16:02:01:01- 16:02:09, 16:10:01- 16:11, 16:14, 16:16-16:23, 16:29, 16:31, 16:35-16:39, 16:41N, 16:43, 16:46, 16:48, 16:50, 16:52-16:53, 16:57-16:58, 16:62N, 16:64- 16:65	
3⁵	210 bp	430 bp	*16:01:01-16:01:16, 16:03-16:04:02, 16:08-16:09:02, 16:13N, 16:15, 16:24-16:25, 16:27- 16:28, 16:30, 16:32-16:34, 16:42, 16:44-16:45, 16:47, 16:49, 16:51, 16:54-16:56, 16:59Q-16:61, 16:63N	
4⁴	115 bp 215 bp 230 bp	430 bp	*16:30 *16:03 *16:46	*15:37:02, 15:57, 15:104:02- 15:104:03
5	220 bp 250 bp	515 bp	*16:04:01-16:04:02, 16:18 *16:41N	*15:21
6	200 bp 280 bp	430 bp	*16:05:01-16:05:02, 16:07 *16:35	*15:10, 15:21

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7	160 bp	515 bp	*16:07	
8⁴	110 bp 175 bp 205 bp 250 bp	430 bp	*16:08 *16:14 *16:26 *16:41N	*15:172
9	140 bp	430 bp	*16:09:01-16:10:02, 16:33, 16:36-16:37, 16:58	*15:01:01:01-15:01:21, 15:01:23- 15:02:09, 15:02:11-15:06:04, 15:08, 15:10, 15:12-15:27, 15:29-15:33, 15:35-15:47, 15:49-15:58, 15:60- 15:68, 15:70-15:87, 15:89-15:95, 15:97-15:114, 15:116-15:129N, 15:131-15:142, 15:144-15:186
10⁴	215 bp 115 bp	430 bp	*16:40 *16:09:01-16:10:02, 16:33, 16:36-16:37, 16:58	*11:01:03, 11:01:10-11:01:11, 11:04:07, 11:08:03, 11:19:02, 11:42:02, 12:01:01:01-12:01:01:06, 12:01:03-12:02:03, 12:02:05-12:10, 12:12-12:15, 12:16:02-12:20, 12:23-12:37, 12:39-12:42, 12:44- 12:48, 12:51-12:52, 12:54-12:56, 12:58-12:65, 12:67-12:72N, 12:74N-12:75, 12:77-12:78, 12:80- 12:84, 13:02:02, 13:42:02, 13:77, 13:163, 13:181, 13:289N, 15:50N, 15:80N, DRB5*01:34-01:35, DRB5*01:41
11	170 bp 210 bp	430 bp	*16:21N *16:11, 16:25	
12	215 bp 240 bp	515 bp	*16:12, 16:17 *16:22	DRB5*01:20, DRB5*01:47, DRB5*01:64, DRB5*02:08, DRB5*02:12, DRB5*02:25N
13⁴	120 bp 165 bp 215 bp	430 bp	*16:19 *16:13N, 16:14 *16:40	
14⁴	85 bp	430 bp	*16:16, 16:27	*11:01:03, 11:01:10-11:01:11, 11:04:07, 11:08:03, 11:19:02, 11:42:02, 12:04, DRB5*01:13, DRB5*01:41
	110 bp 170 bp 240 bp		*16:36-16:37 *16:21N *16:22	DRB5*01:20, DRB5*01:47, DRB5*01:64, DRB5*02:08, DRB5*02:12, DRB5*02:25N
15⁴	80 bp	430 bp	*16:15, 16:23, 16:33-16:34, 16:36, 16:38:01-16:38:02, 16:58	*01:23, 01:111, 04:53:01, 04:99, 11:04:07, 11:42:02, 12:01:01:01- 12:01:01:06, 12:01:03-12:02:03, 12:02:05-12:06, 12:08-12:15, 12:17-12:21, 12:23-12:38, 12:41- 12:48, 12:50-12:65, 12:67-12:72N, 12:74N-12:84, 13:42:02, 13:77, 13:163, 13:181, DRB5*01:31, DRB5*01:44, DRB5*02:08, DRB5*02:12, DRB5*02:26N
16⁶	-	-	Negative Control	

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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*16 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. 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 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 3 may have a tendency to giving rise to primer oligomer formation.

⁶Primer 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.

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	260	200	210	115	220	200	160	110	140	115	170	215
PCR product				215	250	280		175	215		210	240
				230				205				
								250				
Length of int.	515	515	430	430	515	430	515	430	430	430	430	515
pos. control ¹												
5'-primer(s) ²	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	27(167) 5'-CCC 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	47(227) 5'-gTT 3'	14(127) 5'-ggA 3'	6(103) 5'-CAT 3'
											17(136) 5'-ATg 3'	13(125) 5'-gAA 3'
											29(173) 5'-Ag 3'	16(133) 5'-gTA 3'
3'-primer(s) ³	86(344) 5'-CCC 3'	67(286) 5'-gAg 3'	67(286) 5'-gAA 3'	38(200) 5'-gCg 3'	74(307) 5'-CAg 3'	67(286) 5'-gAT 3'	67(286) 5'-gAT 3'	37(197) 5'-CgT 3'	47(227) 5'-ggA 3'	72(303) 5'-gCg 3'	72(303) 5'-gCg 3'	72(303) 5'-gCg 3'
	86(344) 5'-CAC 3'		67(286) 5'-gAA 3'	72(301) 5'-ggC 3'	83(336) 5'-CCT 3'	67(286) 5'-gAT 3'	67(286) 5'-gAT 3'	57(258) 5'-gCT 3'	72(301) 5'-gCC 3'			
			72(301) 5'-ggC 3'	77(317) 5'-AAT 3'		93(364) 5'-CCA 3'		68(289) 5'-CAC 3'				
								83(336) 5'-CCT 3'				
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15
Length of spec.	120	85	80
PCR product	165	110	
	215	170	
		240	
Length of int.	430	430	430
pos. control ¹			
5'-primer(s) ²	13(126) 5'-Agg 3'	6(103) 5'-CAT 3'	72(303) 5'-CgC 3'
		29(173) 5'-Ag 3'	
		50(236) 5'-ggC 3'	
		58(261) 5'-gAg 3'	
3'-primer(s) ³	39(203) 5'-AgT 3'	72(303) 5'-gCg 3'	86(344) 5'-CCA 3'
	52(241) 5'-CTA 3'		
	57(258) 5'-gCT 3'		
	72(301) 5'-gCC 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.

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²The 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.

³The 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.

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¹The 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.

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 alleles to be amplified by primer solutions 4, 6 to 8 and 11 to 14 were available. The specificities of the primers in primer solutions 4, 6, 8 and 14 were tested by separately adding one or two additional 5'-primers, and one additional 3'-primer accordingly. In primer solution 13 it was only possible to test the 5'-primer, the 3'-primers were not possible to be tested. In primer solution 7, 11 and 12 it was only possible to test the 3'-primers, the 5'-primers were not possible to be tested. In primer solution 14, three 5'-primers were not possible to be tested, and in primer solutions 1, 3 to 6, 8 and 9 one, two or three 3'-primer were not possible to be tested.

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Lot-specific information

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