

101.118-24 – including *Taq* polymerase, IFU-01
101.118-24u - without *Taq* polymerase, IFU-02

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“Instructions for Use” (IFU)

Lot No.: **02V**

Lot-specific information

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Olerup SSP® DRB1*07

Product numbers:	101.118-24 – including <i>Taq</i> pol. 101.118-24u – without <i>Taq</i> pol.
Lot number:	02V
Expiry date:	2016-June-01
Number of tests:	24
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. 02V.

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*07 LOT (06R)

The DRB1*07 kit is updated to enable separation of:

- Confirmed DRB1*07 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

A well containing Negative Control primer pairs has been added.

The format of the Product Insert and Worksheet have been changed.

¹As described in section Uniquely Identified Alleles.

The DRB1*07 specificity and interpretation tables have been updated for the DRB1 alleles described since the previous *Olerup SSP®* DRB1*07 lot (**Lot No. 06R**) was made.

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As of lot series V, the Specificity Table is included in the lot-specific Product Insert, and the Interpretation Table is included in the Worksheet.

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

Well	5'-primer	3'-primer	rationale
3	Added	-	5'-primer added for the DRB1*07:26N allele.
4	-	Added	3'-primer added for the DRB1*07:25 allele.
5	-	Added	3'-primer added for the DRB1*07:25
7	-	Added	3'-primer added for the DRB1*07:28 allele.
8	Added	-	5'-primer added for the DRB1*07:26N allele.
9	Added	-	5'-primer added for the DRB1*07:23 allele.
11		Added	3'-primer added for the DRB1*07:24 allele.
12	Added	-	5'-primer added for the DRB1*07:23 allele.
14		Added	3'-primer added from well 16, 3'-primer added for the DRB1*07:27 allele.
15	-	Added	3'-primer added from well 16, 3'-primer added for the DRB1*07:28 allele
16	Moved	Moved	Primers moved to wells 14 and 15, Negative Control.

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Well **16** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup SSP*® HLA Class I, DRB, DQB1 and DPB1 amplicons as well as amplicons generated by a control primer pair.

PCR product sizes range from 75 to 430 base pairs.
The PCR product generated by the control primer pair is 430 base pairs.

Length of PCR product	105	200	105	80	75	80
5'-primer¹	164	340	440	45	45	43
	5'-CAC ^{3'}	5'-Agg ^{3'}	5'-TTA ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}
3'-primer²	231	2nd I	507	59	58	57
	5'-TgC ^{3'}	5'-AAA ^{3'}	5'-TTg ^{3'}	5'-CTC ^{3'}	5'-ggC ^{3'}	5'-CTC ^{3'}
A*	+	+	+			
B*	+	+	+			
C*	+	+	+			
DRB1				+	+	
DRB3				+	+	
DRB5				+		
DQB1					+	
DPB1						+

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*07 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*07:01 to DRB1*07:28 alleles.

PLATE LAYOUT

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

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*07' in silver/gray ink.

Well No. 1 is marked with the Lot No. '02V'.

Wells 1 to 15 – DRB1*07 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

The interpretation of DRB1*07 PCR-SSP subtypings will only be influenced the DRB1*04:90 and 09:08 alleles, when present on the other haplotype. Thus, the interpretation of DRB1*07 subtypings is only marginally influenced by other groups of DRB1 alleles.

UNIQUELY IDENTIFIED ALLELES

All the DRB1*07 alleles, i.e. **DRB1*07:01 to DRB1*07:28**, recognized by the HLA Nomenclature Committee in October 2013^{1,2} will be amplified by the primers in the DRB1*07 subtyping kit.

The DRB1*07 kit enables separation of the confirmed DRB1*07 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*07 alleles is listed below.

The DRB1*07 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.

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The following DRB1*07 alleles can be distinguished by the different sizes of the specific PCR product:

Alleles	Primer mix
DRB1*07:14, 07:24	11
DRB1*07:17, 07:27	14

The DRB1*07 subtyping kit cannot distinguish the silent mutations in the DRB1*07:01:01:01 to DRB1*07:01:07 alleles.

¹DRB1 alleles listed on the IMGT/HLA web page 2013-October-11, release 3.14.0.

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
DRB1*07:01:01:01	Confirmed	DRB1*07:15	Unconfirmed
DRB1*07:01:01:02	Unconfirmed	DRB1*07:16	Unconfirmed
DRB1*07:01:02	Unconfirmed	DRB1*07:17	Unconfirmed
DRB1*07:01:03	Unconfirmed	DRB1*07:18	Unconfirmed
DRB1*07:01:04	Unconfirmed	DRB1*07:19	Unconfirmed
DRB1*07:01:05	Unconfirmed	DRB1*07:20	Confirmed
DRB1*07:01:06	Unconfirmed	DRB1*07:21	Unconfirmed
DRB1*07:01:07	Unconfirmed	DRB1*07:22	Unconfirmed
DRB1*07:03	Confirmed	DRB1*07:23	Unconfirmed
DRB1*07:04	Confirmed	DRB1*07:24	Unconfirmed
DRB1*07:05	Confirmed	DRB1*07:25	Unconfirmed
DRB1*07:06	Unconfirmed	DRB1*07:26N	Unconfirmed
DRB1*07:07	Confirmed	DRB1*07:27	Unconfirmed
DRB1*07:08	Unconfirmed	DRB1*07:28	Unconfirmed
DRB1*07:09	Unconfirmed		
DRB1*07:10N	Unconfirmed		
DRB1*07:11	Confirmed		
DRB1*07:12	Confirmed		
DRB1*07:13	Unconfirmed		
DRB1*07:14	Confirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2013-October-11, release 3.14.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DRB1*07 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

DRB1*07 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for DRB1*07 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB1*07 alleles	Amplified non-DRB1*07 alleles ³
1 ⁵	235 bp	515 bp	*07:01:01:01-07:01:07, 07:03-07:21, 07:23-07:24, 07:26N-07:28	
2	185 bp	430 bp	*07:01:01:01-07:01:07, 07:05-07:10N, 07:12-07:24, 07:26N-07:28	
3	185 bp	430 bp	*07:03, 07:09, 07:26N	
4 ⁴	90 bp 230 bp	515 bp	*07:10N *07:04, 07:25	
5	190 bp 230 bp	430 bp	*07:05 07:11, 07:25	
6	170 bp	430 bp	*07:06	
7	160 bp	430 bp	*07:07, 07:28	
8	175 bp	430 bp	*07:08, 07:26N	
9 ^{4,5}	105 bp 240 bp	430 bp	*07:12 *07:22, 07:23	04:90, 09:08
10	165 bp 220 bp	515 bp	*07:13 *07:18	
11 ⁴	105 bp 260 bp	515 bp	*07:14 *07:24	
12	210 bp 245 bp	430 bp	*07:15, 07:21 07:23	
13	210 bp	430 bp	*07:16, 07:21	
14 ⁴	125 bp 215 bp 260 bp	430 bp	*07:27 *07:17 *07:20	
15	155 bp 220 bp 260 bp	430 bp	*07:19, 07:28 *07:18 *07:20	
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*07 SSP subtypings. 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

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

³Due to sharing of sequence motifs, the DRB1*04:90 and 09:08 alleles are amplified by primer mix 9.

⁴Specific PCR fragments shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR bands.

⁵Primer mixes 1 and 9 may have tendencies of giving rise to primer oligomer artefacts.

⁶Primer mix 16 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by control primer pairs. PCR product sizes range from 75 to 200 base pairs. The PCR product generated by the control primer pair is 430 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. PCR product	235	185	185	90	190	170	160	175	105	165	105	210
				230	230				240	220	260	245
Length of int. pos. control ¹	515	430	430	515	430	430	430	430	430	515	515	430
5'-primer(s) ²	13(127) 5'-ATA 3'	29(174) 5'-AgA 3'	29(173) 5'-AAg 3'	13(127) 5'-ATA 3'	13(127) 5'-ATA 3'	13(127) 5'-ATA 3'	13(127) 5'-ATA 3'	29(173) 5'-AAg 3'	10(116) 5'-gCg 3'	13(127) 5'-ATA 3'	13(127) 5'-ATA 3'	10(116) 5'-gCg 3'
	13(127) 5'-ATA 3'		29(174) 5'-AgT 3'					36(196) 5'-Agg 3'	13(127) 5'-gTA 3'			19(145) 5'-ACC 3'
			29(175) 5'-gAT 3'						56(256) 5'-CTA 3'			21(149) 5'-gAA 3'
3'-primer(s) ³	77(319) 5'-CAC 3'	77(319) 5'-CAC 3'	77(319) 5'-CAC 3'	29(174) 5'-..T 3'	64(278) 5'-TCC 3'	57(257) 5'-CAg 3'	54(248) 5'-gCT 3'	77(319) 5'-CAC 3'	77(319) 5'-CAC 3'	55(251) 5'-gCA 3'	35(191) 5'-CCC 3'	77(319) 5'-CAC 3'
	77(319) 5'-gTA 3'			77(317) 5'-AAT 3'	77(317) 5'-Agg 3'		54(250) 5'-CCA 3'			72(304) 5'-gCT 3'	86(344) 5'-CAA 3'	
				77(319) 5'-CAA 3'	77(319) 5'-CAA 3'							
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15
Length of spec. PCR product	210	125	155
		215	220
		260	260
Length of int. pos. control ¹	430	430	430
5'-primer(s) ²	19(145) 5'-ACC 3'	13(127) 5'-ATA 3'	13(127) 5'-ATA 3'
	22(154) 5'-Agg 3'		
3'-primer(s) ³	77(319) 5'-CAC 3'	42(213) 5'-TCC 3'	49(235) 5'-CAA 3'
		71(301) 5'-CCA 3'	54(248) 5'-gCT 3'
		86(344) 5'-CAT 3'	72(304) 5'-gCT 3'
			86(344) 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 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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CELL LINE VALIDATION SHEET																			
DRB1*07 SSP subtyping kit																			
				Well²															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
				Production No.	201186701	201186702	201325003	201325004	201325005	201186706	201325007	201325008	201325009	201186710	201325011	201325012	201186713	201325014	201325015
IHWC cell line¹	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		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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.

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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 3 to 15 were available. The specificities of the primers in primer solutions 4, 5, 6 and 9 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 3, 8, 12 and 13 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 7, 10, 11 and 14 to 15 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 4 and 5, one 3'-primer was not possible to test, and in primer solution 9, two 5'-primers were not possible to test. In addition, one 3'-primer in primer solution 1 was tested by separately adding one additional 5'-primer.

101.118-24 – including *Taq* polymerase, IFU-01
101.118-24u - without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
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Lot No.: **02V**

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101.118-24u - without *Taq* polymerase, IFU-02

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Lot No.: **02V**

Lot-specific information

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