101.123-24/06 – including *Taq* polymerase, IFU-01
101.123-24u/06u – without *Taq* polymerase, IFU-02

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

Lot No.: 1E2

Lot-specific information

Olerup SSP® DRB5

Product number:	**101.123-24/06 – including *Taq* pol.** **101.123-24u/06u – without *Taq* pol.**
Lot number:	**1E2**
Expiry date:	**2019-02-01**
Number of tests:	**24 test – Product No. 101.123-24/24u** **6 tests – Product No. 101.123-06/06u**
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. 1E2.

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

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® DRB5 LOT (79Y)

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

The DRB5 specificity and interpretation tables have been updated for the HLA-DRB alleles described since the previous *Olerup* SSP® DRB5 lot was made **(Lot No. 79Y)**. The kit design is based on IMGT/HLA database 3.24.0.

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	-	Modified	3'-primer modified for improved HLA-specific amplification.
7	Added	-	5'-primer added for the DRB5*01:17 allele.
8	-	Added	3'-primer added from well 14 for the DRB5*01:09 allele.

101.123-24/06 – including *Taq* polymerase, IFU-01
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Lot No.: 1E2 **Lot-specific information**

9	Added	Added	Primer pair added for the DRB5*01:16 allele.
11	-	Added	3'-primer added from well 14 for the DRB5*01:14 allele.
13	Added	Added	Primer pair added for the DRB5*01:18 allele.
14	Removed, Added	Moved, Added	Primer pair added for the DRB5*02:07 allele, 3'-primers moved to well 8 and 11.

Lot No.: 1E2 **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.

Lot No.: 1E2 **Lot-specific information**

PRODUCT DESCRIPTION

DRB5 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB5*01:01:01 to DRB5*01:18 and the DRB5*02:02 to DRB5*02:07 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 'DRB5' in silver/gray ink.
Well No. 1 is marked with the Lot No. '1E2'.
Wells 1 to 15 – DRB5 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

Only alleles of the DRB5 locus will be amplified by the DRB5 subtyping kit, except for a few DRB1, DRB4, DRB7 and DRB8 alleles that will be amplified by primer mixes 1 to 3, 13 and 15.
For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the DRB5 alleles, i.e. **DRB5*01:01:01 to DRB5*01:18 and DRB5*02:02 to DRB5*02:07**, recognized by the HLA Nomenclature Committee in April 2016[1,2] will give rise to unique amplification patterns by the primers in the DRB5 subtyping kit.

[1]DRB5 alleles listed on the IMGT/HLA web page 2016-04-15, release 3.24.0, 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.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DRB5 homo- and heterozygotes is available upon request.

101.123-24/06 – including *Taq* polymerase, IFU-01
101.123-24u/06u – without *Taq* polymerase, IFU-02

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Lot No.: 1E2

Lot-specific information

SPECIFICITY TABLE

DRB5 SSP subtyping

Specificities and sizes of the PCR products of the 15+1 primer mixes used for DRB5 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified DRB5 alleles[3]	Other amplified DRB alleles[4]
1	255 bp	**515 bp**	*01:01:01-01:05, 01:07-01:18, 02:03	**DRB1*09:07**
2	210 bp	**515 bp**	*01:01:01-01:05, 01:07-01:10N, 01:12-01:18, 02:04	**DRB1*09:07**
3	225 bp	430 bp	*01:01:01-01:02, 01:04-01:05, 01:07-01:10N, 01:12-01:18, 02:05	**DRB1*09:07**
4[5]	100 bp 150 bp	**515 bp**	*01:01:01-01:01:03, 01:04, 01:06-01:07, 01:09, 01:11, 01:15-01:18 *02:06	
5	150 bp	**515 bp**	*01:01:01, 01:05, 01:07, 01:09, 01:13, 01:16-01:18	
6	145 bp	430 bp	*01:02-01:03, 01:05, 01:08N, 01:10N	
7	145 bp	430 bp	*01:02-01:03, 01:08N, 01:10N, 01:17, 02:05	
8	215 bp	430 bp	*01:03, 01:06, 01:09, 01:11, 02:02-02:04, 02:06-02:07	
9[5]	85 bp 175 bp 225 bp	430 bp	*01:16 *01:13 *01:04	
10	130 bp 160 bp	430 bp	*01:07 *01:12, 01:15	
11[5]	110 bp 200 bp	430 bp	*01:14 *01:06, 01:11, 02:02-02:03, 02:06-02:07	
12	185 bp	**515 bp**	*02:02, 02:04-02:07	
13	150 bp 195 bp	430 bp	*01:01:02?, 01:03?, 01:07?, 01:09?, 01:18, 02:04? *01:08N	**DRB1*15:02:03?, DRB1*15:86, DRB1*16:01:02?, DRB1*16:02:02?, DRB1*16:05:01?, DRB4*01:05?, DRB4*01:07?, DRB7*01:01:02?, DRB8*01:01:01:01?**
14	145 bp	430 bp	*02:07	
15	235 bp	430 bp	*01:10N, 01:12, 01:15	**DRB1*09:07**
16[6]	-	-	**Negative Control**	

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For *In Vitro* Diagnostic Use.

101.123-24/06 – including *Taq* polymerase, IFU-01
101.123-24u/06u – without *Taq* polymerase, IFU-02

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Lot No.: 1E2 **Lot-specific information**

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

[3]For several DRB alleles 1st and/or 3rd exon(s) and above, 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 and that unknown sequences of codons 87 to 92 are identical with the DRB1*01:01 consensus sequence.

[4]Due to the sharing of sequence motifs between DRB alleles the DRB1*09:07 allele will be amplified by primer mixes 1 to 3, 13 and 15.

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

[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.

'?', nucleotide sequence information not available for the primer matching sequence.

101.123-24/06 – including *Taq* polymerase, IFU-01
101.123-24u/06u – without *Taq* polymerase, IFU-02

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Lot No.: 1E2 **Lot-specific information**

101.123-24/06 – including *Taq* polymerase, IFU-01
101.123-24u/06u – without *Taq* polymerase, IFU-02

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Lot No.: 1E2

Lot-specific information

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8
Length of spec.	255	210	225	100	150	145	145	215
PCR product(s)				150				
Length of int.	515	515	430	515	515	430	430	430
pos. control[1]								
5'-primer(s)[2]	13(125)	13(125)	13(125)	37(199)	36(196)	37(199)	36(196)	13(125)
	5' -gTA 3'	5' -gTA 3'	5' -gTA 3'	5' -ACT 3'	5' -Agg 3'	5' -ACg 3'	5' -AgA 3'	5' -gTA 3'
				97(379)		37(199)	41(209)	
				5' -CTg 3'		5' -ACg 3'	5' -Cgg 3'	
3'-primer(s)[3]	85(341)	66(286)	71(299)	57(258)	72(303)	72(303)	69(295)	69(295)
	5' -CAA 3'	5' -gAA 3'	5' -gCC 3'	5' -gCg 3'	5' -gCg 3'	5' -gCg 3'	5' -CTg 3'	5' -gTT 3'
		66(286)	73(307)	134(490)			72(303)	71(299)
		5' -gAA 3'	5' -CAg 3'	5' -gCC 3'			5' -gCg 3'	5' -gCg 3'
		70(296)	77(319)					71(299)
		5' -TCC 3'	5' -CAC 3'					5' -gCg 3'
		72(303)						
		5' -gCg 3'						
Well No.	1	2	3	4	5	6	7	8

Well No.	9	10	11	12	13	14	15
Length of spec.	85	130	110	185	150	145	235
PCR product(s)	175	160	200		195		
	225						
Length of int.	430	430	430	515	430	430	430
pos. control[1]							
5'-primer(s)[2]	13(125)	37(199)	13(125)	36(196)	57(258)	23(157)	13(125)
	5' -gTA 3'	5' -ACT 3'	5' -gTA 3'	5' -AgA 3'	5' -gAC 3'	5' -ggT 3'	5' -gTA 3'
	120(446)				107(409)		
3'-primer(s)[3]	5' -gAC 3'				5' -AgA 3'		
	58(260)	66(286)	36(196)	85(341)	93(365)	58(261)	77(319)
	5' -CCT 3'	5' -gAT 3'	5' -gTA 3'	5' -CAg 3'	5' -gCg 3'	5' -TCA 3'	5' -CAC 3'
	73(307)	77(319)	66(286)		159(565)		79(323)
	5' -CAg 3'	5' -CAC 3'	5' -gAT 3'		5' -CAT 3'		5' -TgC 3'
	134(490)						
	5' -gCT 3'						
Well No.	9	10	11	12	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.

Lot No.: 1E2 **Lot-specific information**

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 or the 3 terminal nucleotides of the primer is given.

101.123-24/06 – including *Taq* polymerase, IFU-01
101.123-24u/06u – without *Taq* polymerase, IFU-02

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Lot No.: 1E2 **Lot-specific information**

CELL LINE VALIDATION SHEET

DRB5 SSP subtyping kit

				Well[2]														
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
			Prod. No.:	201556401	201556402	201669803	201556404	201556405	201556406	201669807	201669808	201669809	201556410	201669811	201556412	201669813	201669814	201556415
	IHWC cell line[1]		DRB5															
1	9001	SA		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*01:02	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-
3	9011	E4181324	*01:02	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-
4	9275	GU373		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*02:02	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-
6	9353	SM		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025	DEU		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*01:01	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

101.123-24/06 – including *Taq* polymerase, IFU-01
101.123-24u/06u – without *Taq* polymerase, IFU-02

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Lot No.: 1E2 **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 alleles to be amplified by primer solutions 9, 10, 14 and 15 were available. The specificities of the primers in primer solutions 9, 10 and 15 were tested by separately adding two or three additional 5'-primers, respectively one or two additional 3'-primers. In primer solution 14 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solutions 4, 7 and 9 one 5'-primer was not possible to test, and in primer solutions 2, 8, 13 and 15 one 3'-primer was not possible to test. Additional primers in primer solutions 1 to 4, 7, 8, 11 and 13 were tested by separately adding additional 5'-primers or 3'-primers.

Lot No.: 1E2 **Lot-specific information**

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