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

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Lot No.: 25V

Lot-specific information

Olerup SSP® DRB5

Product number:	**101.123-24/06 – including *Taq* pol.** **101.123-24u/06u – without *Taq* pol.**
Lot number:	**25V**
Expiry date:	**2016-July-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 -20°C**
- PCR Master Mix:	**-20°C**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 25V.

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(49R)

A well containing Negative Control primer pairs has been added.

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. 49R)**.

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
7	--	Added	3'-primer added from well 16.
16	Moved	Moved	Primer pair moved to well 7, Negative Control.

Change in revision R01 compared to R00:

1. Primer mix 7 does amplify the DRB5*02:05 allele. This has been corrected in the Specificity table.

Lot No.: 25V **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 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'-primer[1]	**164**	**340**	**440**	**45**	**45**	**43**
	5'-CAC3'	**5'-Agg3'**	**5'-TTA3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-Tgg3'**
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**
A*	**+**	**+**	**+**			
B*	**+**	**+**	**+**			
C*	**+**	**+**	**+**			
DRB1				**+**	**+**	
DRB3				**+**	**+**	
DRB5				**+**		
DQB1					**+**	
DPB1						**+**

[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 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.

[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.

101.123-24/06 – including *Taq* polymerase, IFU-01
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Lot No.: 25V **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*01:14 and the DRB5*02:02 to DRB5*02:06 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. '25V'.
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 that the DRB1*09:07 allele will be amplified by primer mixes 1 to 3 and 15.

UNIQUELY IDENTIFIED ALLELES

All the DRB5 alleles, i.e. **DRB5*01:01:01 to DRB5*01:14 and DRB5*02:02 to DRB5*02:06**, recognized by the HLA Nomenclature Committee in October 2013[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 2013-October-11, release 3.14.0, 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.

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

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Lot No.: 25V

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[3] alleles	Other amplified DRB alleles[4]
1	255 bp	**515 bp**	*01:01:01-01:05, 01:07-01:14, 02:03	**DRB1*09:07**
2	210 bp	**515 bp**	*01:01:01-01:05, 01:07-01:10N, 01:12-01:14, 02:04	**DRB1*09:07**
3[6]	225 bp	430 bp	*01:01:01-01:02, 01:04-01:05, 01:07-01:10N, 01:12-01:14, 02:05	**DRB1*09:07**
4[5,7]	100 bp 150 bp	430 bp	*01:01:01-01:01:02, 01:04, 01:06-01:07, 01:09, 01:11 *02:06	
5	150 bp	**515 bp**	*01:01:01, 01:05, 01:07, 01:09, 01:13	
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, 02:05	
8	215 bp	430 bp	*01:03, 01:06, 01:11, 02:02-02:04, 02:06	
9	175 bp 225 bp	430 bp	*01:13 *01:04	
10	130 bp 160 bp	430 bp	*01:07 *01:12	
11	200 bp	430 bp	*01:06, 01:11, 02:02-02:03, 02:06	
12	185 bp	**515 bp**	*02:02, 02:04-02:06	
13	195 bp	430 bp	*01:08N	
14[5]	110 bp 210 bp	430 bp	*01:14 *01:09	
15	240 bp	430 bp	*01:10N, 01:12	**DRB1*09:07**
16[8]	-	-	**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 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.

Lot No.: 25V **Lot-specific information**

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 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 3 frequently gives rise to an extra band longer than the control band. This band should be disregarded in the interpretation of DRB5 SSP typings.

[7]Primer mix 4 has a tendency to giving rise to primer oligomer formation.

[8]Primer 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.

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

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Lot No.: 25V

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	430	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)		
				5' -CTg 3'		5' -ACg 3'		
3'-primer(s)[3]	85(341)	66(286)	71(299)	57(258)	72(303)	72(303)	69(295)	71(299)
	5' -CAA 3'	5' -gAA 3'	5' -gCC 3'	5' -gCg 3'	5' -gCg 3'	5' -gCg 3'	5' -CTg 3'	5' -gCg 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)					
		5' -TCC 3'	5' -CAC 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.	175	130	200	185	195	110	240
PCR product(s)	225	160				210	
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)	107(409)	13(125)	13(125)
	5' -gTA 3'	5' -ACT 3'	5' -gTA 3'	5' -AgA 3'	5' -AgA 3'	5' -gTA 3'	5' -gTA 3'
3'-primer(s)[3]	58(260)	66(286)	66(286)	85(341)	159(565)	36(196)	77(319)
	5' -CCT 3'	5' -gAT 3'	5' -gAT 3'	5' -CAg 3'	5' -CAT 3'	5' -gTA 3'	5' -CAC 3'
	73(307)	77(319)				69(295)	79(323)
	5' -CAg 3'	5' -CAC 3'				5' -gTT 3'	5' -TgC 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. 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.: 25V **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.:	201297401	201297402	201297403	201209604	201297405	201297406	201329207	201297408	201297409	201297410	201297411	201297412	201297413	201297414	201297415
	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 Visit www.olerup-ssp.com for
101.123-24u/06u – without *Taq* polymerase, IFU-02 **"Instructions for Use" (IFU)**

Lot No.: 25V **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, 14 and 15 were tested by separately adding one or two additional 5'-primer, respectively one or two additional 3'-primer(s). In primer solution 4 one 5'-primer was not possible to test, and in primer solutions 2, 14 and 15 one 3'-primer was not possible to test. Additional 3'-primers in primer solutions 1 to 4, 7, 8 and 11 were tested by separately adding additional 5'-primers.

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Lot No.: 25V **Lot-specific information**

ADDRESSES:

Manufacturer:
Olerup SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.
Tel: +46-8-717 88 27
Fax: +46-8-717 88 18
E-mail: info-ssp@olerup.com
Web page: http://www.olerup-ssp.com

Distributed by:
Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.
Tel: +43-1-710 15 00
Fax: +43-1-710 15 00 10
E-mail: support-at@olerup.com
Web page: http://www.olerup.com

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382
Tel: 1-877-OLERUP1
Fax: 610-344-7989
E-mail: info.us@olerup.com
Web page: http://www.olerup.com

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.