

101.122-24/06 – including *Taq* pol., IFU-01
101.122-24u/06u – without *Taq* pol., IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **67S**

Lot-specific information

Olerup SSP® DRB4

Product number:	101.122-24/06 – including <i>Taq</i> pol. 101.122-24/06u – without <i>Taq</i> pol.
Lot number:	67S
Expiry date:	2016-February-01
Number of tests:	24 test – Product No. 101.122-24 6 tests – Product No. 101.122-06
Number of wells per test:	13
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. 67S.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP® DRB4 LOT (86N)

The DRB4 specificity and interpretation tables have been updated for the DRB alleles described since the previous *Olerup SSP®* DRB4 lot was made (Lot No. 86N).

The Lot-specific information for DRB4 including and without *Taq* polymerase is now described in one common Product Insert.

The DRB4 primer set is unchanged compared to the previous lot.

Change in revision R01 compared to R00:

1. Primer mix 4 does not amplify the DRB4*03:01N allele. This has been corrected in the Specificity and Interpretation Tables.

Change in revision R02 compared to R01:

1. Primer mix 8 may give rise to a lower yield of HLA-specific PCR product than the other DRB4 primer mixes. A foot note has been added in the Specificity Table.

Change in revision R03 compared to R02:

1. The genotype of the 9025/DEU cell line is DRB4*01:03. This has been corrected in the Cell Line Validation Sheet.

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PRODUCT DESCRIPTION

DRB4 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB4*01:01:01:01 to DRB4*03:01N alleles.

PLATE LAYOUT

Each test consists of 13 PCR reactions in a 16 well cut PCR plate. Wells 14 to 16 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	empty	empty	empty

The 16 well cut PCR plate is marked with 'DRB4' in silver/gray ink.

Well No. 1 is marked with the Lot No. '67S'.

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 DRB4 alleles will be amplified by the primers in the DRB4 SSP subtyping kit¹. Thus, the interpretation of DRB4 SSP subtypings is not influenced by alleles of other DRB genes.

¹The DRB1*15:01:01:01-15:94 and the DRB1*16:01:01-16:05:02, 16:07-16:18 and 16:20-16:23 alleles might be faintly amplified by primer mix 7.

UNIQUELY IDENTIFIED ALLELES

All the DRB4 alleles, i.e. **DRB4*01:01:01:01 to DRB4*03:01N**, recognized by the HLA Nomenclature Committee in April 2013¹ will give rise to unique amplification patterns by the primers in the DRB4 subtyping kit.

The DRB4 subtyping kit cannot distinguish the silent mutation in the DRB4*01:03:01:01, 01:03:01:03-01:03:04 alleles.

¹DRB4 alleles listed on the IMGT/HLA web page 2013-April-17, release 3.12.0, www.ebi.ac.uk/imgt/hla.

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RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 15 alleles generate 11 amplification patterns that can be combined in 66 homozygous and heterozygous combinations. 8 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products were not considered in these calculations.

+-----+ ----- *01:01, *01:01 = *01:01, *03:01N
+-----+ ----- *01:01, *01:06 = *01:06, *03:01N
+-----+ ----- *01:01, *01:03:01:01 = *01:03:01:01, *03:01N
+-----+ ----- *01:01, *01:03:01:02N = *01:03:01:02N, *03:01N

*01:03:01:01 = *01:03:01:01 and 01:03:02- 01:03:04

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SPECIFICITY TABLE

DRB4 SSP subtyping

Specificities and sizes of the PCR products of the 13 primer mixes used for DRB4 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB4 ³ alleles
1	185 bp	515 bp	*01:01:01:01, 01:03:01:01-01:03:04, 01:05-01:08
2⁶	140 bp	430 bp	*01:02
3	130 bp	430 bp	*01:01:01:01, 01:04 [?] -01:05 [?] , 01:06, 01:07 [?] -01:08 [?] , 02:01N, 03:01N
4	245 bp	515 bp	*01:01:01:01-01:03:01:01, 01:03:01:03-01:04, 01:05 [?] , 01:06-01:08, 02:01N
5⁵	155 bp	430 bp	*01:03:01:02N
6	190 bp	430 bp	*01:04
7⁷	155 bp	430 bp	*01:02-01:03:04, 01:04 [?] -01:05 [?] , 01:07 [?] -01:08 [?]
8⁸	290 bp	515 bp	*01:01:01:01, 01:04 [?] -01:08 [?] , 02:01N [?] , 03:01N
9⁶	155 bp	515 bp	*01:05
10⁴	85 bp	515 bp	*02:01N
11⁴	110 bp	430 bp	*01:06
12	210 bp	430 bp	*01:07
13	215 bp	430 bp	*01:08

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 DRB4 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 respective lengths of the HLA-specific PCR product(s) are given for the alleles amplified by these primer mixes.

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 two different control primer pairs give rise to either an internal positive control band of 430

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base pairs, for most wells, or a band of 515 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB4 subtyping.

In addition, wells number 4, 8, 9 and 10 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

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

³The nucleotide sequences of the 3rd exon of the DRB4*01:04, DRB4*01:05, DRB4*01:07 and DRB4*01:08 alleles are not yet available. Thus, it is not known whether the DRB4*01:04, DRB4*01:05, DRB4*01:07 and DRB4*01:08 alleles will be amplified by primer mix 3 or 7.

The complete 2nd exon nucleotide sequence of the DRB4*01:05 allele is not known. Thus, it is not known whether the DRB4*01:05 allele will be amplified by primer mix 4 or not.

Second intron sequences of the DRB4*01:04 to DRB4*01:08 and DRB4*02:01N alleles are not known. Thus, it is not known whether these alleles will be amplified by primer mix 8 or not.

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

⁵Primer mix 5 may have tendencies of unspecific amplifications.

⁶Primer mixes 2 and 9 have a tendency to giving rise to primer oligomer formation

⁷The DRB1*15:01:01:01-15:94 and the DRB1*16:01:01-16:05:02, 16:07-16:18 and 16:20-16:23 alleles might be faintly amplified by primer mix 7.

⁸Primer mix 8 may give rise to a lower yield of HLA-specific PCR product than the other DRB4 primer mixes.

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

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INTERPRETATION TABLE								
DRB4 SSP subtyping								
Amplification patterns of the DRB4 alleles								
	Well							
	1	2	3	4	5	6	7⁹	8
Length of spec.	185	140	130	245	155	190	155	290
PCR product								
Length of int.	515	430	430	515	430	430	430	515
pos. control¹								
5'-primer²	28(170)	42(213)	105(401)	1 st I	1 st I	28(170)	96(375)	2 nd I
	5' -gAT 3'	5' -AgT 3'	5' -AAA 3'	5' -ggg 3'	5' -CAA 3'	5' -gAT 3'	5' -CAA 3'	5' -TgA 3'
3'-primer³	76(314)	76(314)	134(490)	1st I	42(213)	77(317)	134(490)	2nd I
	5' -TgT 3'	5' -TgC 3'	5' -gCT 3'	5' -TgC 3'	5' -TCA 3'	5' -AgT 3'	5' -gCC 3'	5' -TTC 3'
Well No.	1	2	3	4	5	6	7⁹	8
DRB4 allele¹⁰								
*01:01:01:01	1		3	4				8
*01:02		2		4			7	
*01:03:01:01, 01:03:01:03-01:03:04	1			4			7	
*01:03:01:02N	1				5		7	
*01:04			?	4		6	?	?
*01:05	1		?	?			?	?
*01:06	1		3	4				?
*01:07	1		?	4			?	?
*01:08	1		?	4			?	?
*02:01N			3	4				?
*03:01N			3					8
DRB4 allele								
Well No.	1	2	3	4	5	6	7⁹	8

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INTERPRETATION TABLE					
DRB4 SSP subtyping					
Amplification patterns of the DRB4 alleles					
Well					
9	10	11	12	13	
155	85	110	210	215	Length of spec.
					PCR product
515	515	430	430	430	Length of int.
					pos. control¹
42(213)	28(170)	111(421)	28(170)	28(170)	5'-primer²
5' -AgT 3'	5' -gAT 3'	5' -ACT 3'	5' -gAT 3'	5' -gAT 3'	
80(328)	42(213)	134(490)	83(337)	86(346)	3'-primer³
5' -gTg 3'	5' -TCA 3'	5' -gCT 3'	5' -CCg 3'	5' -CTT 3'	
9	10	11	12	13	Well No.
					DRB4 allele¹⁰
					*01:01:01:01
					*01:02
					*01:03:01:01,
					01:03:01:03-01:03:04
					*01:03:01:02N
					*01:04
9					*01:05
		11			*01:06
			12		*01:07
				13	*01:08
	10				*02:01N
					*03:01N
					DRB4 allele
9	10	11	12	13	Well No.

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB4 subtyping.

In addition, wells number 4, 8, 9 and 10 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

²The codon, and in parenthesis the nucleotide, in the 2nd exon unless otherwise noted, matching the specificity-determining 3'-end of the primer is given. Codon and 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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³The codon, and in parenthesis the nucleotide, in the 2nd exon unless otherwise noted, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Codon and 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.

⁴Matching sequences within the 3rd exon.

⁵Matching sequences within the 1st intron.

⁶Matching sequences from the 3'-end of the 1st intron into the 5'-end of the 2nd exon.

⁷Matching the sequence of the 3'-end of the 1st intron.

⁸Matching sequences within the 2nd intron.

⁹The DRB1*15:01:01:01-15:94 and the DRB1*16:01:01-16:05:02, 16:07-16:18 and 16:20-16:23 alleles might be faintly amplified by primer mix 7.

¹⁰The DRB4*01010102N allele has been renamed DRB4*01:03:01:02N.

'?' The nucleotide sequences of the 3rd exon of the DRB4*01:04, DRB4*01:05, DRB4*01:07 and DRB4*01:08 alleles are not yet available. Thus, it is not known whether the DRB4*01:04, DRB4*01:05, DRB4*01:07 and DRB4*01:08 alleles will be amplified by primer mix 3 or 7.

The complete 2nd exon nucleotide sequence of the DRB4*01:05 allele is not known. Thus, it is not known whether the DRB4*01:05 allele will be amplified by primer mix 4 or not.

Second intron sequences of the DRB4*01:04 to DRB4*01:08 and DRB4*02:01N alleles are not known. Thus, it is not known whether these alleles will be amplified by primer mix 8 or not.

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CELL LINE VALIDATION SHEET													
DRB4 SSP kit													
						Well							
						1	2	3	4	5	6	7	8
					Prod. No.:	201202701	201202702	201202703	201202704	201202705	201202706	201202707	201202708
						201202709	201202710	201202711	201202712	201202713			
	IHWC cell line	DRB4											
1	9001 SA					-	-	-	-	-	-	-	-
2	9280 LK707	*01:03				+	-	-	+	-	-	+	-
3	9011 E4181324					-	-	-	-	-	-	f	-
4	9275 GU373					-	-	-	-	-	-	-	-
5	9009 KAS011					-	-	-	-	-	-	f	-
6	9353 SM	*01:03				+	-	-	+	-	-	+	-
7	9020 QBL					-	-	-	-	-	-	-	-
8	9025 DEU	*01:03				+	-	-	+	-	-	+	-
9	9026 YAR	*01:03				+	-	-	+	-	-	+	-
10	9107 LKT3	*01:03				+	-	-	+	-	-	+	-
11	9051 PITOUT	*01:01				+	-	+	+	-	-	-	+
12	9052 DBB	*01:03N				+	-	-	-	+	-	+	-
13	9004 JESTHOM					-	-	-	-	-	-	-	-
14	9071 OLGA					-	-	-	-	-	-	-	-
15	9075 DKB	*01:03				+	-	-	+	-	-	+	-
16	9037 SWEIG007					-	-	-	-	-	-	-	-
17	9282 CTM3953540					-	-	-	-	-	-	-	-
18	9257 32367	*01:01				+	-	+	+	-	-	-	+
19	9038 BM16					-	-	-	-	-	-	-	-
20	9059 SLE005					-	-	-	-	-	-	-	-
21	9064 AMALA					-	-	-	-	-	-	-	-
22	9056 KOSE					-	-	-	-	-	-	-	-
23	9124 IHL					-	-	-	-	-	-	-	-
24	9035 JBUSH					-	-	-	-	-	-	-	-
25	9049 IBW9	*01:01				+	-	+	+	-	-	-	+
26	9285 WT49					-	-	-	-	-	-	-	-
27	9191 CH1007	*01:03				+	-	-	+	-	-	+	-
28	9320 BEL5GB	*01:01				+	-	+	+	-	-	+	+
29	9050 MOU	*01:01				+	-	+	+	-	-	-	+
30	9021 RSH					-	-	-	-	-	-	-	-
31	9019 DUCAF					-	-	-	-	-	-	-	-
32	9297 HAG					-	-	-	-	-	-	-	-
33	9098 MT14B	*01:03				+	-	-	+	-	-	+	-
34	9104 DHIF					-	-	-	-	-	-	-	-
35	9302 SSTS	*01:03				+	-	-	+	-	-	+	-
36	9024 KT17	*01:03				+	-	-	+	-	-	+	-
37	9065 HHKB					-	-	-	-	-	-	-	-
38	9099 LZL					-	-	-	-	-	-	-	-
39	9315 CML	*01:02				-	+	-	+	-	-	+	-
40	9134 WHONP199	*01:03				+	-	-	+	-	-	+	-
41	9055 H0301					-	-	-	-	-	-	-	-
42	9066 TAB089					-	-	-	-	-	-	-	-
43	9076 T7526	*01:03				+	-	-	+	-	-	+	-
44	9057 TEM					-	-	-	-	-	-	-	-
45	9239 SHJO	*01:01	*01:03			+	-	+	+	-	-	+	+
46	9013 SCHU					-	-	-	-	-	-	f	-
47	9045 TUBO					-	-	-	-	-	-	-	-
48	9303 TER-ND					-	-	-	-	-	-	-	-

† The DRB1*15:01:01:01-15:94 and the DRB1*16:01:01:16:05:02, 16:07-16:18 and 16:20-16:23 alleles might be faintly amplified by primer mix 7.

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CERTIFICATE OF ANALYSIS

Olerup SSP® DRB4 SSP

Product number: 101.122-24/06 – including *Taq* pol.
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Lot number: 67S
Expiry date: 2016-February-01
Number of tests: 24 test – Product No. 101.122-24
6 tests – Product No. 101.122-06
Number of wells per test: 13

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2012-027-01	9	2012-027-09
2	2012-027-02	10	2012-027-10
3	2012-027-03	11	2012-027-11
4	2012-027-04	12	2012-027-12
5	2012-027-05	13	2012-027-13
6	2012-027-06		
7	2012-027-07		
8	2012-027-08		

The specificity of each primer solution of the kit has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 6 and 9 to 13 were available. The specificities of the primers in primer solutions 6 and 9 were tested by separately adding one additional 5'-primer, respectively, one additional 3'-primer. In primer solutions 10 and 11 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 12 and 13 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. One additional 3'-primer in primer solution 1 was tested by adding one additional 5'-primer.

Results: No false positive or false negative amplifications were obtained.

Date of approval: 2013-August-23

Approved by:

Production Quality Control

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Declaration of Conformity

Product name: *Olerup* SSP® DRB4
Product number: 101.122-24/24u, -06/06u
Lot number: 67S

Intended use: DRB4 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Franzengatan 5
SE-112 51 Stockholm, Sweden
Phone: +46-8-717 88 27
Fax: +46-8-717 88 18

We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2008 and ISO 13485:2012, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, conformity assessed using Annex IV, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at *Olerup* SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom. (Notified Body number: 0088.)

Stockholm, Sweden
2013-August-23

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

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Lot-specific information

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