

101.122-24/06 – including *Taq* pol., IFU-01 Rev. No. 03
101.122-24u/06u – without *Taq* pol., IFU-02 Rev. No. 03

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

Lot No.: **86N**

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:	86N
Expiry date:	2014-December-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. 86N.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP[®] DRB4 LOT(01L)

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. 01L).

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 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.

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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. ‘86N’.

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*15:01:01:01-15:67 and 15:70-15:71 and the DRB1*16:01:01-16:05:02 and 16:07-16:18 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 2012¹ 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 and 01:03:01:03-01:03:04 alleles.

¹DRB4 alleles listed on the IMGT/HLA web page 2012-April-12, release 3.8.0, 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. 6 of these genotypes do not give rise to unique amplification patterns.

+--+-----	-----	*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

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

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Lot-specific information

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 alleles ³
1	185 bp	515 bp	*01:01, 01:03:01:01-01:03:04, 01:05-01:08
2⁷	140 bp	430 bp	*01:02
3	130 bp	430 bp	*01:01, 01:04 [?] -01:05 [?] , 01:06, 01:07 [?] -01:08 [?] , 02:01N, 03:01N
4	245 bp	515 bp	*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
7⁶	155 bp	430 bp	*01:02-01:03:04, 01:04 [?] -01:05 [?] , 01:07 [?] -01:08 [?]
8⁸	290 bp	515 bp	*01:01, 01:04 [?] -01:08 [?] , 02:01N [?] , 03:01N
9⁷	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 specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective lengths of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

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.

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Lot No.: 86N**Lot-specific information**

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.

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.

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

⁵Primer mix 5 may have tendencies of unspecific amplifications.

⁶The DRB1*15:01:01:01-15:67 and 15:70-15:71 and the DRB1*16:01:01-16:05:02 and 16:07-16:18 alleles might be faintly amplified by primer mix 7.

⁷Primer mixes 2 and 9 might have tendencies of primer dimer formation.

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. PCR product	185	140	130	245	155	190	155	290
Length of int. pos. control¹	515	430	430	515	430	430	430	515
5'-primer²	28(170)	42(213)	105(401) ⁴	1 st I ⁵	1 st I ⁷	28(170)	96(375)	2 nd I ⁸
	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) ⁴	5 ⁶	42(213)	77(317)	134(490) ⁴	2 nd I ⁹
	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	7⁸	8
DRB4 allele¹⁰								
*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	7⁸	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)⁴	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)⁴	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
					*01:02
					*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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Lot No.: 86N**Lot-specific information**

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 to DRB1*15:51, DRB1*16:01:01 to DRB1*16:05:02 and DRB1*16:07 to DRB1*16:17 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	9	10	11	12	13
				Prod. No.:	201202701	201202702	201202703	201202704	201202705	201202706	201202707	201202708	201202709	201202710	201202711	201202712	201202713
	IHCW 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	*01:01		+	-	+	+	-	-	-	+	-	-	-	-	-
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	SSTO	*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 to DRB1*15:51, DRB1*16:01:01 to DRB1*16:05:02 and DRB1*16:07 to DRB1*16:17 alleles might be faintly amplified by primer mix 7.

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Lot-specific information

CERTIFICATE OF ANALYSIS

Olerup SSP® DRB4 SSP

Product number: 101.122-24/06 – including *Taq* pol.
101.122-24/06u – without *Taq* pol.
Lot number: 86N
Expiry date: 2014-December-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: 2012-June-29

Approved by:

Production Quality Control

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Lot-specific information

Declaration of Conformity

Product name: *Olerup* SSP® DRB4
Product number: 101.122-24/24u, -06/06u
Lot number: 86N

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 13485:2003, 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
2012-June-29

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

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Lot-specific information

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