

101.331.24/06 – including *Taq* pol., IFU-01 Rev. No. 03
101.331.24u/06u – without *Taq* pol., IFU-02 Rev. No. 03

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

Lot No.: **13N**

Lot-specific information
Olerup SSP[®] DPA1

Product number:	101.331-24/06 – including <i>Taq</i> pol. 101.331-24u/06u – without <i>Taq</i> pol.
Lot number:	13N
Expiry date:	2014-July-01
Number of tests:	24 test – Product No. 101.331-24/24u 6 tests – Product No. 101.331-06/06u
Number of wells per test:	16
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. 13N.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP[®] DPA1 LOT

The DPA1 specificity and interpretation tables have been updated for the DPA1 alleles described since the previous *Olerup SSP[®] DPA1* lot was made (**Lot No. 53K**).

The Lot-specific information for DPA1 including and without *Taq* polymerase is now described in one common Product Insert.

The DPA1 primer set is unchanged compared to previous lot

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PRODUCT DESCRIPTION

DPA1 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DPA1*01:03 to DPA1*04:01 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	16

The 16 well cut PCR plate is marked with ‘DPA1’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘13N’.

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 heat-sealed with a PCR-compatible foil.

Please note: When removing each 16 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Only DPA1 alleles will be amplified by the DPA1 typing kit. Thus, the interpretation of DPA1 typings is not influenced by the DPA2 gene.

UNIQUELY IDENTIFIED ALLELES

All the phenotypically different DPA1 alleles, i.e. **DPA1*01:03 to DPA1*01:10**, **DPA1*02:01 to DPA1*02:04**, **DPA1*03:01 to DPA1*03:03** and **DPA1*04:01**, recognized by the HLA Nomenclature Committee in January 2012¹ will give rise to unique amplification patterns by the primers in the DPA1 typing kit.

The DPA1 typing kit cannot distinguish the following silent mutations: DPA1*01:03:01:01, DPA1*01:03:02 and DPA1*01:03:04, the DPA1*01:06:01 and DPA1*01:06:02, the DPA1*02:01:01 to DPA1*02:01:07 and the DPA1*02:02:01 to DPA1*02:02:04..

¹DPA1 alleles listed on the IMGT/HLA web page 2012-January-12, release 3.7.0, www.ebi.ac.uk/imgt/hla.

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RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 33 alleles generate 17 amplification patterns that can be combined in 153 homozygous and heterozygous combinations. 23 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.

++++-----	--+-----+	*01:03:01:01, *03:03 = *01:04, *03:01
+++-----	-----	*01:05, *01:06:01 = *01:10, *02:01:01
+++-----	+-----	*01:03:01:01, *02:04 = *01:10, *02:02:01 = *01:10, *02:04
+++-----	-----	*01:03:01:01, *01:06:01 = *01:06:01, *01:10
+++-----	-----	*01:03:01:01, *01:10 = *01:10, *01:10
+++-----	-----	*01:03:01:01, *01:07 = *01:07, *01:07
+++-----	-----	*01:03:01:01, *01:09 = *01:09, *01:09
+++-----	-----	*01:04, *01:08 = *01:08, *01:08
+-----	-----	*01:05, *04:01 = *04:01, *04:01
-+++-----	+-----	*01:06:01, *02:02:01 = *01:06:01, *02:04
-----	+-----	*02:02:01, *02:04 = *02:04, *02:04

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

*01:06:01 = *01:06:01-01:06:02

*02:01:01 = *02:01:01-02:01:07

*02:02:01 = *02:02:01-02:02:04

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Lot-specific information
SPECIFICITY TABLE

DPA1 SSP typing

Specificities and sizes of the PCR products of the 16 primer mixes used for DPA1 SSP typing

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DPA1 ³ alleles
1^{4,5}	85 bp	515 bp	*01:03:01:01-01:03:02, 01:03:04-01:05, 01:07-01:10, 04:01
2	255 bp	515 bp	*01:03:01:01-01:04, 01:06:01-01:10
3	205 bp	430 bp	*01:03:01:01-01:03:04, 01:06:01-01:07, 01:09-01:10, 03:01-03:02
4^{4,6}	115 bp	430 bp	*01:04, 01:08, 03:03
5⁴	105 bp	430 bp	*01:05, 02:01:01-02:04, 04:01
6⁹	155 bp, 195 bp	515 bp	*01:06:01-01:06:02, 01:10, 02:04
7⁴	100 bp	430 bp	*01:06:01-01:06:02, 02:01:01-02:01:07
8⁴	100 bp	430 bp	*02:02:01-02:02:04, 02:04
9	205 bp	430 bp	*02:02:01-02:02:04, 02:04, 03:02
10^{4,8}	85 bp	515 bp	*01:03:03, 02:03, 03:01-03:03
11^{4,7,8}	90 bp	515 bp	*03:01, 03:03
12	205 bp	430 bp	*04:01
13	135 bp	430 bp	*01:07
14	140 bp	430 bp	*01:08
15⁸	245 bp	430 bp	*01:03:03, 03:01-03:03
16	220 bp	430 bp	*01:09

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 DPA1 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

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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 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 DPA1 typing.

In addition, wells number 2, 6, 10 and 11 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.

³For some DPA1 alleles only partial 2nd exon nucleotide sequences are 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. We assume that unknown sequences are conserved within allelic groups.

⁴Specific PCR fragments shorter than 125 bp are less intense and less sharp compared to longer specific PCR fragments.

⁵Primer mix 1 may give rise to primer dimer formation.

⁶Primer mix 4 may faintly amplify the DPA1*04:01 allele.

⁷Primer mix 11 may give rise to non-specific amplification.

⁸Primer mixes 10, 11 and 15 may give a lower yield of specific PCR product than the other DPA1 primer mixes.

⁹Primer mix 6: Specific PCR fragment of 155 bp in the DPA1*01:10 and 02:04 alleles. Specific PCR fragment of 195 bp in the DPA1*01:06:01-01:06:02 alleles.

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INTEPRETATION TABLE								
DPA1 SSP typing								
Amplification patterns of the DPA1 alleles								
	Well ⁵							
	1	2	3	4	5	6	7	8
Length of spec.	85	255	205	115	105	155	100	100
PCR product(s)						195		
Length of int.	515	515	430	430	430	515	430	430
pos. control ¹								
5'-primer(s) ²	15 (138) 5'-ACg 3'	11 (125) 5'-CgC 3'	28 (177) 5'-gAA 3'	4 (103) 5'-Cgg 3'	84 (345) 5'-AAT 3'	31 (185) 5'-gCA 3'	11 (125) 5'-CgC 3'	11 (125) 5'-CAT 3'
						43 (222) 5'-TgT 3'		
3'-primer(s) ³	31 (184) 5'-CAT 3'	83 (340) 5'-ggT 3'	83 (340) 5'-ggT 3'	28 (177) 5'-TCg 3'	2 nd I 5'-ggC 3'	69 (298) 5'-gTC 3'	31 (184) 5'-CTg 3'	31 (184) 5'-CTg 3'
						83 (340) 5'-ggT 3'		
Well No.	1	2	3	4	5	6	7	8
DPA1 allele ⁴								
*01:03:01-01:03:02, 01:03:04	1	2	3					
*01:03:03		2	3					
*01:04	1	2		4				
*01:05	1				5			
*01:06:01-01:06:02		2	3			6	7	
*01:07	1	2	3					
*01:08	1	2		4				
*01:09	1	2	3					
*01:10	1	2	3			6		
*02:01:01-02:01:07					5		7	
*02:02:01-02:02:04					5			8
*02:03					5			
*02:04					5	6		8
*03:01			3					
*03:02			3					
*03:03				4				
*04:01	1				5			
DPA1 allele ⁴								
Well No.	1	2	3	4	5	6	7	8

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INTERPRETATION TABLE								
DPA1 SSP typing								
Amplification patterns of the DPA1 alleles								
Well ⁵								
9	10	11	12	13	14	15	16	
205	85	90	205	135	140	245	220	Length of spec. PCR product(s)
430	515	515	430	430	430	430	430	Length of int. pos. control ¹
11 (125) 5'-CAT 3'	15 (138) 5'-ACC 3'	66 (290) 5'-ATC 3'	18 (145) 5'-gAA 3'	51 (244) 5'-AAA 3'	50 (242) 5'-CCg 3'	15 (138) 5'-ACC 3'	23 (161) 5'-TAC 3'	5'-primer(s) ²
66 (290) 5'-TCA 3'	31 (184) 5'-CAT 3'	83 (340) 5'-ggT 3'	73 (310) 5'-AgC 3'	83 (340) 5'-ggT 3'	83 (340) 5'-ggT 3'	83 (340) 5'-ggT 3'	83 (340) 5'-ggT 3'	3'-primer(s) ³
9	10	11	12	13	14	15	16	Well No. DPA1 allele ⁴
								*01:03:01-01-01:03:02, 01:03:04
	10					15		*01:03:03
								*01:04
								*01:05
								*01:06:01-01:06:02
				13				*01:07
					14			*01:08
							16	*01:09
								*01:10
								*02:01:01-02:01:07
9								*02:02:01-02:02:04
	10							*02:03
9								*02:04
	10	11				15		*03:01
9	10					15		*03:02
	10	11				15		*03:03
			12					*04:01
								DPA1 allele ⁴
9	10	11	12	13	14	15	16	Well No.

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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 DPA1 typing.

In addition, wells number 2, 6, 10 and 11 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, 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.

³The codon, and in parenthesis the nucleotide, in the 2nd exon and in the 2nd intron, 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.

⁴The sequence of the DPA1*0101 allele has been shown to be identical to DPA1*01:03:01.

The sequence of the DPA1*0102 allele has been shown to be identical to DPA1*01:03:01.

⁵Primer mix 6: Specific PCR fragment of 155 bp in the DPA1*01:10 and 02:04 alleles. Specific PCR fragment of 195 bp in the DPA1*01:06:01-01:06:02 alleles.

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CELL LINE VALIDATION SHEET																				
DPA1 SSP kit																				
				Well																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Lot No.:	200968201	200968202	201077403	200968204	200968205	200968206	200968207	200968208	200968209	200968210	200968211	200968212	200968213	200968214	200968215	200968216
	IHWC cell line	DPA1																		
1	9001 SA	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707	*01:03		-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-
3	9011 E4181324	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373	*02:01	*04:01	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-
5	9009 KAS011	*01:03	*02:01	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
6	9353 SM	*02:02		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025 DEU	*01		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3	*02:02		-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-
11	9051 PITOUT	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540	*01:03	*02:01	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*01:03	*03:01	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	+	-
19	9038 BM16	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*01:03	*02:01	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*02:01		-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*01:03	*04:01	+	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-
28	9320 BEL5GB	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*02:02	*03:01	-	-	+	-	+	-	-	+	+	+	+	-	-	-	+	-	-
31	9019 DUCAF	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*02:02		-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-
37	9065 HHKB	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML	*01:03	*02:01	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*02:02		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*02:01		-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*02:02		-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-
43	9076 T7526	*04:01		+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-
44	9057 TEM	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*01:03	*03:01	+	+	+	-	-	-	-	-	-	-	+	+	-	-	-	+	-
46	9013 SCHU	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-



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CERTIFICATE OF ANALYSIS

Olerup SSP® DPA1 SSP

Product number: 101.331-24/06 – including *Taq* pol.
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Lot number: 13N
Expiry date: 2014-July-01
Number of tests: 24 test – Product No. 101.331-24/24u
 6 tests – Product No. 101.331-06/06u
Number of wells per test: 16

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2009-682-01	9	2009-682-09
2	2009-682-02	10	2009-682-10
3	2010-774-03	11	2009-682-11
4	2009-682-04	12	2009-682-12
5	2009-682-05	13	2009-682-13
6	2009-682-06	14	2009-682-14
7	2009-682-07	15	2009-682-15
8	2009-682-08	16	2009-682-16

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

No DNAs carrying the allele to be amplified by primer solutions 6, 13, 14 and 16 were available. The specificities of the primers in primer solutions 6 and 14 were tested by separately adding one additional 5'-primer and one additional 3'-primer, respectively. In primer solutions 13 and 16 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solution 6, one 5'-primer and one 3'-primer was not possible to test.

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

Date of approval: 2012-January-30

Approved by:

Approved by:

Production Quality Control

101.331.24/06 – including *Taq* pol., IFU-01 Rev. No. 03
101.331.24u/06u – without *Taq* pol., IFU-02 Rev. No. 03

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“Instructions for Use” (IFU)

Lot No.: **13N**

Lot-specific information

Declaration of Conformity

Product name: *Olerup* SSP[®] DPA1
Product number: 101.331-24/24u, -06/06u
Lot number: 13N

Intended use: HLA-DPA1 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 III, 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.

Stockholm, Sweden
2012-January-30

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

101.331.24/06 – including **Taq pol.**, IFU-01 Rev. No. 03
101.331.24u/06u – without **Taq pol.**, IFU-02 Rev. No. 03

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