Olerup SSP® DPA1

Product number:	**101.331-24/06 – including *Taq* pol.**
Lot number:	**53K**
Expiry date:	**2012-September-01**
Number of tests:	**24 test – Product No. 101.331-24**
	6 tests – Product No. 101.331-06
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. 53K.

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. 73G)**.

The DPA1 primer set is unchanged compared to previous lot (**73G**).

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. '53K'.
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 July 2010[1] will give rise to unique amplification patterns by the primers in the DPA1 typing kit.
The DPA1 typing kit cannot distinguish the DPA1*01:03:01, DPA1*01:03:02 and DPA1*01:03:04 alleles, the DPA1*01:06:01 and DPA1*01:06:02 alleles, the DPA1*02:01:01 to DPA1*02:01:07 alleles and the DPA1*02:02:01 to DPA1*02:02:03 alleles.

[1]DPA1 alleles listed on the IMGT/HLA web page 2010-July-16, release 3.1.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 28 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, *03:03 = *01:04, *03:01
+++-+++- --------	*01:05, *01:06:01 = *01:10, *02:01:01
+++-+++ +-------	*01:03:01, *02:04 = *01:10, *02:02:01 = *01:10, *02:04
+++-++- --------	*01:03:01, *01:06:01 = *01:06:01, *01:10
+++-+-- --------	*01:03:01, *01:10 = *01:10, *01:10
+++----- ----+---	*01:03:01, *01:07 = *01:07, *01:07
+++----- -------+	*01:03:01, *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 = *02:02:01-02:02:03

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**	*01:03:01-01:03:02, 01:03:04-01:05, 01:07-01:10, 04:01
2	255 bp	**515 bp**	*01:03:01-01:04, 01:06:01-01:10
3	205 bp	430 bp	*01:03:01-01:03:04, 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
5[4]	105 bp	430 bp	*01:05, 02:01:01-02:04, 04:01
6[9]	155 bp, 195 bp	**515 bp**	*01:06:01-01:06:02, 01:10, 02:04
7[4]	100 bp	430 bp	*01:06:01-01:06:02, 02:01:01-02:01:07
8[4]	100 bp	430 bp	*02:02:01-02:02:03, 02:04
9	205 bp	430 bp	*02:02:01-02:02:03, 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
15	245 bp	430 bp	*01:03:03, 03:01-03:03
16	220 bp	430 bp	*01:09

[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 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 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 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.
[3]For 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.
[4]Specific PCR fragments shorter than 125 bp are less intense and less sharp compared to longer specific PCR fragments.
[5]Primer mix 1 may give rise to primer dimer formation.
[6]Primer mix 4 may faintly amplify the DPA1*04:01 allele.
[7]Primer mix 11 may give rise to non-specific amplification.
[8]Primer mixes 10 and 11 may give a lower yield of specific PCR product than the other DPA1 primer mixes.
[9]Primer 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 and DPA1*01:06:02 alleles.

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)**	**11(125)**	**28(177)**	**4(103)**	**84(345)**	**31(185)**	**11(125)**	**11(125)**
	5' -ACg 3'	**5' -CgC 3'**	**5' -gAA 3'**	**5' -Cgg 3'**	**5' -AAT 3'**	**5' -gCA 3'**	**5' -CgC 3'**	**5' -CAT 3'**
						43(222)		
						5' -TgT 3'		
3'-primer(s)[3]	**31(184)**	**83(340)**	**83(340)**	**28(177)**	**2nd I**	**69(298)**	**31(184)**	**31(184)**
	5' -CAT 3'	**5' -ggT 3'**	**5' -ggT 3'**	**5' -TCg 3'**	**5' -ggC 3'**	**5' -gTC 3'**	**5' -CTg 3'**	**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**	
***02:02:01-02:02:03**					**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**

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]
								*01:03:01-01:03:02, 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								*02:02:01-02:02:03
	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.

[1]The 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.
[2]The 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.
[3]The codon, and in parenthesis the nucleotide, in the 2nd exon, 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.
[4]The 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.
[5]Primer 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 and DPA1*01:06:02 alleles.

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		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® DPA1 SSP

Product number: **101.331-24/06 – including *Taq* pol.**
Lot number: **53K**
Expiry date: **2012-September-01**
Number of tests: **24 test – Product No. 101.331-24**
6 tests – Product No. 101.331-06
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: 2010-November-24

Approved by:

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DPA1
Product number: 101.331-24/06
Lot number: 53K

Intended use: HLA-DPA1 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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SE-133 33 Saltsjöbaden, 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 Construction File is maintained at *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

Saltsjöbaden, Sweden
2010-November-24

Olle Olerup
Managing Director

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