Olerup SSP® DPA1

Product number:	**101.331-24/06 – including *Taq* pol.**
Lot number:	**20F**
Expiry date:	**2010-October-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. 20F.

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. 11E)**.

The primers of the Wells detailed below have been added, exchanged or modified.

Well	5'-primer	3'-primer	rationale
6	Added	-	Primer added for the DPA1*0110 allele.

PRODUCT DESCRIPTION

DPA1 SSP subtyping

CONTENT
The primer set contains 5'- and 3'-primers for identifying the DPA1*0103 to DPA1*0401 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 in.
Well No. 1 is marked with the Lot No. '20F'.
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*0103 to DPA1*0110, DPA1*0201 to DPA1*0204, DPA1*0301 to DPA1*0303 and DPA1*0401**[1], recognized by the HLA Nomenclature Committee in October 2008[2] will give rise to unique amplification patterns by the primers in the DPA1 typing kit.
The DPA1 typing kit cannot distinguish the DPA1*010301, DPA1*010302, DPA1*010304 alleles, the DPA1*020101 to DPA1*020106 alleles and the DPA1*020201 and DPA1*020203 alleles.

[1]The nucleotide sequence of the DPA1*010602 allele is not yet retrievable.
[2]DPA1 alleles listed on the IMGT/HLA web page 2008-October-10, release 2.23.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 16 DPA1 alleles give rise to 17 different amplification patterns, that can be combined in 153 homozygous and heterozygous combinations. Twenty-three of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products generated by primer mix 6 were not considered in these calculations.

```
++++---- -++---+-     0103,0303 = 0104,0301
+++-+++- --------     0105,0106 = 0110,0201
+++-++-+ +-------     0103,0204 = 0110,0202 = 0110,0204
+++--++- --------     0103,0106 = 0106,0110
+++--+-- --------     0103,0110 = 0110,0110
+++----- ----+---     0103,0107 = 0107,0107
+++----- -------+     0103,0109 = 0109,0109
++-+---- -----+--     0104,0108 = 0108,0108
+---+--- ---+----     0105,0401 = 0401,0401
-++-++++ +-------     0106,0202 = 0106,0204
----++-+ +-------     0202,0204 = 0204,0204

0103 = 010301 and 010302 and 010304
```

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]	85 bp	**515 bp**	010301-010302, 010304, 0104, 0105, 0107-0110, 0401
2	250 bp	**515 bp**	010301-0104, 010601, 0107-0110
3	200 bp	430 bp	010301-010304, 010601, 0107, 0109, 0110, 0301, 0302
4[4]	115 bp	430 bp	0104, 0108, 0303
5[4]	105 bp	430 bp	0105, 020101-0204, 0401
6[5]	155, 195 bp	**515 bp**	010601, 0110, 0204
7[4]	100 bp	430 bp	010601, 020101-020106
8[4]	100 bp	430 bp	020201-020203, 0204
9	205 bp	430 bp	020201-020203, 0204, 0302
10[4]	85 bp	**515 bp**	010303, 0203, 0301-0303
11[4]	90 bp	**515 bp**	0301, 0303
12	205 bp	430 bp	0401
13	135 bp	430 bp	0107
14	140 bp	430 bp	0108
15	245 bp	430 bp	010303, 0301-0303
16	220 bp	430 bp	0109

[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 length 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.
The nucleotide sequence of the DPA1*010602 allele is not yet retrievable.
[4]Specific PCR fragments shorter than 125 bp are less intense and less sharp compared to longer specific PCR fragments.
[5]Primer mix 6: Specific PCR fragment of 155 bp in the DPA1*0110 and 0204 alleles. Specific PCR fragment of 195 bp in the DPA1*010601 allele.

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**	**250**	**200**	**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**	**11**	**28**	**4**	**84**	**31**	**11**	**11**
	5'-ACg3'	**5'-C gC3'**	**5'-gAA3'**	**5'-Cg g3'**	**5'-AAT3'**	**5'-g CA3'**	**5'-C gC3'**	**5'-C AT3'**
						43		
						5'-TgT3'		
3'-primer(s)[3]	**31**	**83**	**83**	**28**	**intr[6]**	**69**	**31**	**31**
	5'-CAT3'	**5'-ggT3'**	**5'-ggT3'**	**5'-TC g3'**	**5'-ggC3'**	**5'-gTC3'**	**5'-CTg3'**	**5'-CTg3'**
						83		
						5'-ggT3'		
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
DPA1 allele[4]								
***010301-010302, 010304**	**1**	**2**	**3**					
***010303**		**2**	**3**					
***0104**	**1**	**2**		**4**				
***0105**	**1**				**5**			
***010601**		**2**	**3**			**6**	**7**	
***0107**	**1**	**2**	**3**					
***0108**	**1**	**2**		**4**				
***0109**	**1**	**2**	**3**					
***0110**	**1**	**2**	**3**			**6**		
***020101-020106**					**5**		**7**	
***020201-020203**					**5**			**8**
***0203**					**5**			
***0204**					**5**	**6**		**8**
***0301**			**3**					
***0302**			**3**					
***0303**				**4**				
***0401**	**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	15	66	18	51	50	15	23	5'-primer(s)[2]
5'-C AT3'	5'-ACC3'	5'-A TC3'	5'-gA A3'	5'-CA T3'	5'-C Cg3'	5'-ACC3'	5'-T AC3'	
	15					15		
	5'-ACC3'					5'-ACC3'		
66	31	83	73	83	83	83	83	3'-primer(s)[3]
5'-T CA3'	5'-CAT3'	5'-ggT3'	5'-AgC3'	5'-ggT3'	5'-ggT3'	5'-ggT3'	5'-ggT3'	
9	10	11	12	13	14	15	16	Well No.
								DPA1 allele[4]
								*010301-010302, 010304
	10					15		*010303
								*0104
								*0105
								*010601
				13				*0107
					14			*0108
							16	*0109
								*0110
								*020101-020106
9								*020201-020203
	10							*0203
9								*0204
	10	11				15		*0301
9	10					15		*0302
	10	11				15		*0303
			12					*0401
								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, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given. 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. Empty spaces indicate codon boundaries.
[3]The codon, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. 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. Empty spaces indicate codon boundaries.
[4]The sequence of the DPA1*0101 allele has been shown to be identical to DPA1*0103.
The sequence of the DPA1*0102 allele has been shown to be identical to DPA1*0103.
The nucleotide sequence of the DPA1*010602 allele is not yet retrievable.
[5]Primer mix 6: Specific PCR fragment of 155 bp in the DPA1*0110 and 0204 alleles. Specific PCR fragment of 195 bp in the DPA1*010601 allele.
[6]'intr', matching sequences in the 2nd intron.

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.:	200730601	200730602	200730603	200730604	200730605	200852306	200730607	200730608	200730609	200730610	200730611	200730612	200730613	200730614	200730615	200841316
	IHWC cell line		DPA1																	
1	9001	SA	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*0201	*0202	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-
3	9011	E4181324	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*0201	*0401	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-
5	9009	KAS011	*0103	*0201	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-
6	9353	SM	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9007	DEM	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*0202		-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-
11	9051	PITOUT	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9067	BTB	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9008	WILJON	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*0103	*0301	+	+	+	-	-	-	-	-	-	+	+	-	-	-	+	-
19	9038	BM16	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*0103	*0201	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-
23	9124	IHL	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*0201		-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
26	9285	WT49	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*0103	*0401	+	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-
28	9320	BEL5GB	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*0202	*0301	-	-	+	-	+	-	-	+	+	+	+	-	-	-	+	-
31	9019	DUCAF	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*0202		-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-
37	9065	HHKB	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*0103	*0201	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*0201		-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*0202		-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-
43	9076	T7526	*0401		+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
44	9057	TEM	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*0103	*0301	+	+	+	-	-	-	-	-	-	+	+	-	-	-	+	-
46	9013	SCHU	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*0103		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® DPA1 SSP

Product number: **101.331-24/06 – including *Taq* pol.**
Lot number: **20F**
Expiry date: **2010-October-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	2007-306-01	9	2007-306-09
2	2007-306-02	10	2007-306-10
3	2007-306-03	11	2007-306-11
4	2204-306-04	12	2007-306-12
5	2007-306-05	13	2007-306-13
6	2008-523-06	14	2007-306-14
7	2007-306-07	15	2007-306-15
8	2007-306-08	16	2008-413-16

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.

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

Date of approval: 2009-May-26

Approved by:

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DPA1
Product number: 101.331-24/06
Lot number: 20F

Intended use: HLA-DPA1 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Hasselstigen 1
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 9001:2000, ISO 17025:1999 and ISO 13485:2000, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex III.

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
2009-May-26

Olle Olerup
Managing Director

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