Lot-specific information

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Olerup SSP[®] DQB1*06:02,DQA1*01:02 - SSP

Product number:	101.901-24 – including <i>Taq</i> polymerase
Lot number:	96K
Expiry date:	2013-July-01
Number of tests:	24
Number of wells per test:	8
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. 96K.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP® DQB1*06:02,DQA1*01:02 LOT

The DQB1*06:02,DQA1*01:02 specificity and interpretation tables have been updated for the DQB1 and DQA1 alleles described since the previous *Olerup* SSP[®] DQB1*06:02,DQA1*01:02 lot **(Lot No. 17G)** was made.

The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot.

Well	5'-primer	3'-primer	rationale
7	Added	-	Primers added for the DQB1*06:23 and *06:37
			alleles.
8	-	Added	Primers added for the DQB1*06:14:01-
			06:14:02 and *06:15 alleles.

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PRODUCT DESCRIPTION

DQB1*06:02,DQA1*01:02 - SSP

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DQB1*06:02 and DQA1*01:02 alleles.

Please note that DQB1 amplifications usually are somewhat less pronounced than e.g. DRB and DQA1 amplifications even when using the same DNA preparation and exactly the same experimental procedures.

PLATE LAYOUT

Each test consists of 8 PCR reactions dispensed in an 8 well cut PCR plate

1	2	3	4	5	6	7	8
							~

The 8 well PCR plate is marked with '96K' in silver/gray ink.

Well No. 1 is marked with the Lot No. '96K'.

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 8 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

The DQB1*03:30 allele and seven DQB1*04 alleles will be amplified by the primers in vial 6. Thus, the interpretation is only marginally influenced by other groups of DQB1 alleles, and not by the DQB2 and DQB3 genes.

Only DQA1*01 alleles will be amplified by the primers in vials 3, 4 and 5. Thus, the interpretation is not influenced by other groups of DQA1 alleles or the DQA2 gene.

UNIQUELY IDENTIFIED ALLELES

All the DQB1*06 alleles, i.e. **DQB1*06:01 to DQB1*06:39**, and all the DQA1*01 alleles, i.e. **DQA1*01:01 to DQA1*01:07**, recognized by the HLA Nomenclature Committee in October 2010¹ have been considered in the specificity and interpretation tables of the DQB1*06:02,DQA1*01:02 primer set.

The DQB1*06:02,DQA1*01:02 kit cannot distinguish the DQB1*06:02:01-06:02:02 alleles or the DQA1*01:02:01-01:02:04 alleles.

¹DQB1 and DQA1 alleles listed on the IMGT/HLA web page 2010-October-15, release 3.2.0, <u>www.ebi.ac.uk/imgt/hla</u>.

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SPECIFICITY TABLE

DQB1*06:02,DQA1*01:02 - SSP

Specificities and sizes of the PCR products of the 8 primer mixes used for DQB1*06:02,DQA1*01:02 SSP typing

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified alleles ³
1	210 bp	515 bp	DQB1*06:01:01-06:02:02, 06:05:02 [?] -06:06 [?] , 06:10-06:11:02, 06:13, 06:16, 06:18-06:20, 06:24, 06:29, 06:33, 06:35, 06:37
2	185 bp	430 bp	DQB1*06:02:01-06:02:02, 06:14:01-06:16, 06:19-06:20, 06:23-06:24, 06:33, 06:37
3 ⁵	170 bp	430 bp	DQA1*01:01:01-01:02:04, 01:04:01-01:07
4	145 bp	430 bp	DQA1*01:02:01-01:03, 01:06
5 ⁴	95 bp	430 bp	DQA1*01:06
6 ⁶	155 bp, 195 bp	430 bp	DQB1*06:01:01 ^w -06:01:06 ^w , 06:16, 06:19, DQB1*03:30, 04:01:01-04:03:02, 04:06
7 ^{4,8}	115 bp, 225 bp, 265 bp	430 bp	DQB1*06:20, 06:23, 06:31, 06:33, 06:37
8 ^{4,5,8}	60 bp, 175 bp, 220 bp	430 bp	DQB1*06:03:01-06:05:01, 06:05:02 [?] -06:06 [?] , 06:07:01-06:09, 06:12, 06:14:01-06:15, 06:17, 06:21-06:22, 06:24-06:28, 06:30- 06:32, 06:34, 06:36, 06:38-06:39

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 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.

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 DQB1*06:02,DQA1*01:02 subtyping.

³Due to sharing of sequence motifs, the DQB1*03:30 as well as some DQB1*04 allele are amplified by primer mix 6.

⁴Short specific PCR fragments are less intense and not as sharp as longer specific bands.

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⁵Primer mixes 3 and 8 may give rise to a primer dimer artifact. ⁶Primer mix 6: Specific PCR product of 155 bp in the DQB1*06:01:01^w-06:01:06^w and 06:19 and in the DQB1*03:30, 04:01:01-04:03:02 and 04:06 alleles. Specific PCR product of 195 bp in the DQB1*06:16 allele.

⁷Primer mix 7: Specific PCR product of 115 bp in the DQB1*06:37 allele. Specific PCR product of 225 bp in the DQB1*06:23 allele. Specific PCR product of 265 bp in the DQB1*06:20, 06:31 and 06:33 alleles.

⁸Primer mix 8: Specific PCR product of 60 bp in the DQB1*06:03:01-06:03:02, 06:08:01-06:08:02, 06:14:01-06:14:02, 06:26-06:28, 06:31-06:32

allele.Specific PCR product of 175 bp in the DQB1*06:24 alleles. Specific PCR product of 220 bp in the DQB1*06:05:01, 06:05:02?-06:06?, 06:09, 06:12, 06:15, 06:22 allele. Specific PCR product of 60 and 175 bp the DQB1*06:30 allele. Specific PCR product of 60 and 220 bp in the DQB1*06:04:01-06:04:03, 06:07:01-06:07:02, 06:21, 06:25, 06:34, 06:36, 06:38-06:39 allele. Specific PCR product of 60, 175 and 220 bp in the DQB1*06:17 allele. All specific bands may not always be visible.

??, nucleotide sequence of the primer matching region is not available for this allele.

'w', might be weakly amplified.

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INTERPRETA	TION ⁻	TABL	E			
DQB1*06	:02 SS	6P				
	Well ⁴					
	1	2		6	7	8
Length of spec.	210	185		155	115	60
PCR product				195	225	175
					265	220
Length of int.	515	430		430	430	430
pos. control ¹						
5'-primer ²	30(184)	9(122)		9(122)	11(129)	26(173)
	^{5'} -gAT 3'	^{5'} -gTT ^{3'}		^{5'} -gTT ^{3'}	^{5'} -TTA ^{3'}	^{5'} -TCT ^{3'}
	30(184)				14(136)	
	^{5'} -gAT ^{3'}				^{5'} -gCC ^{3'}	
					26(173)	
					^{5'} -ggg ^{3'}	
					62(282)	
					^{5'} -AAg ^{3'}	
3'-primer ³	86(353)	57(266)		47(237)	87(356)	30(184)
	^{5'} -ACg ^{3'}	^{5'} -CAT ^{3'}	:	^{5'} -CgA ^{3'}	^{5'} -ggA ^{3'}	^{5'} -gTg ^{3'}
				60(274)		71(307)
				^{5'} -gTT ^{3'}		^{5'} -ggC ^{3'}
						86(353)
						^{5'} -ACC ³
Well No.	1	2		6	7	8
DQB1 allele						
DQB1*06:01:01-06:01:06	1			w		
DQB1*06:02:01-06:02:02	1	2				
DQB1*06:03:01-06:05:01, 06:07:01-06:09,						
06:12, 06:17, 06:21-06:22, 06:25-06:28,						8
06:30, 06:32, 06:34, 06:36, 06:38-06:39						
DQB1*06:05:02-06:06	?					?
DQB1*06:10-06:11:02, 06:13, 06:18,	1					
06:29, 06:35						
DQB1*06:14:01-06:15		2				8
DQB1*06:16, 06:19	1	2		6		
DQB1*06:20, 06:33, 06:37	1	2			7	
DQB1*06:23		2			7	
DQB1*06:24	1	2				8
DQB1*06:31					7	8
DQB1*03:30, 04:01:01-04:03:02, 04:06				6		
DQB1 allele						
Well No.	1	2		6	7	8

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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 DQB1*06:02,DQA1*01:02 subtyping.

²The codon, and in parenthesis the nucleotide, in the 2nd exon, matching the specificitydetermining 3'-end of the primer is given. Codon and nucleotide numbering as on the <u>www.ebi.ac.uk/imgt/hla</u> 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, matching the specificitydetermining 3'-end of the primer is given. Codon and nucleotide numbering as on the <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given.

⁴Primer mix 6: Specific PCR product of 155 bp in the DQB1*06:01:01^w-06:01:06^w and 06:19 and in the DQB1*03:30, 04:01:01-04:03:02 and 04:06 alleles. Specific PCR product of 195 bp in the DQB1*06:16 allele.

Primer mix 7: Specific PCR product of 115 bp in the DQB1*06:37 allele. Specific PCR product of 225 bp in the DQB1*06:23 allele. Specific PCR product of 265 bp in the DQB1*06:20, 06:31 and 06:33 alleles.

Primer mix 8: Specific PCR product of 60 bp in the DQB1*06:03:01-06:03:02, 06:08:01-06:08:02, 06:14:01-06:14:02, 06:26-06:28, 06:31-06:32

allele.Specific PCR product of 175 bp in the DQB1*06:24 alleles. Specific PCR product of 220 bp in the DQB1*06:05:01, 06:05:02?-06:06?, 06:09, 06:12, 06:15, 06:22 allele. Specific PCR product of 60 and 175 bp the DQB1*06:30 allele. Specific PCR product of 60 and 220 bp in the DQB1*06:04:01-06:04:03, 06:07:01-06:07:02, 06:21, 06:25, 06:34, 06:36, 06:38-06:39 allele. Specific PCR product of 60, 175 and 220 bp in the DQB1*06:17 allele. All specific bands may not always be visible.

"?", nucleotide sequence of the primer matching region is not available for this allele.

w', might be weakly amplified.

IFU-01 Rev. No. 02 can be downloaded from

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Lot No.: 96K
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Lot-specific information

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INTERPRETATION TABLE						
DQA1*01:02 SSP						
Well						
	3	4	5			
Length of spec.	170	145	95			
PCR product						
Length of int.	430	430	430			
pos. control ¹						
5'-primer ²	25(143)	34(169)	25(143)			
	^{5'} -gTA 3'	^{5'} -AgC ^{3'}	^{5'} -gTA ^{3'}			
3'-primer ³	69(274)	69(274)	44(199)			
	^{5′} -TgC ^{3′}	⁵ ' -TgC ^{3'}	^{5'} -AgC ^{3'}			
Well No.	3	4	5			
DQA1 allele						
DQA1*01:01:01-01:01:02,	3					
01:04:01-01:05, 01:07	5					
DQA1*01:02:01-01:02:04	3	4				
DQA1*01:03		4				
DQA1*01:06	3	4	5			
DQA1 allele						
Well No.	3	4	5			

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 DQB1*06:02,DQA1*01:02 subtyping. ²The codon, and in parenthesis the nucleotide, in the 2nd exon, matching the specificity-determining 3'-end

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 <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given.

sequence of the 3 terminal nucleotides of the primer is given. ³The 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 <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given.

"?", nucleotide sequence of the primer matching region is not available for this allele.

'w', might be weakly amplified.

Product Insert

IFU-01 Rev. No. 02 can be downloaded from

Lot No.: **96K**

Lot-specific information

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[]	CELL LINE VALIDATION SHEET									
	DQB1*06:02									
						We	<u>.</u>			
					1	2		6	7	8
										-
					200962601	200962602		200962606	201081507	508
				2	8	62(62	81	81
					2009626(600		600	10	201081
					L X	20		20	20	20
		<u>/C cell line</u>	D	<u>QB1</u>						
1	9001		*05:01		-	-		-	-	-
2	9280	LK707	*06:01	*02:02	+	-		w	-	-
3	9011	E4181324	*06:01		+	-		w	-	-
4		GU373	*02:01		-	-		-	-	-
5	9009	KAS011	*05:02		-	-		-	-	-
6	9353		*03:02	*06:01	+	-		w	-	-
7	9020		*02:01		-	-		-	-	-
8	9025		*03:01		-	-		-	-	-
9	9026	YAR	*03:02		-	-		-	-	-
10	9107	LKT3	*04:01		-	-		+	-	-
11		PITOUT	*02:02		-	-		-	-	-
12	9052	DBB	*03:03		-	-		-	-	-
13	9004	JESTHOM	*05:01		-	-		-	-	-
14		OLGA	*04:02		-	-		+	-	-
15	9075		*03:03		-	-		-	-	-
16	9037	SWEIG007	*03:01		-	-		-	-	-
17		CTM3953540	*02:01	*06:03	-	-		-	-	+
18	9257	32367	*06:02	*02:02	+	+		-	-	-
19		BM16	*03:01		-	-		-	-	-
20	9059	SLE005	*06:04		-	-		-	-	+
21		AMALA	*03:01		-	-		-	-	-
22		KOSE	*05:03	*06:04	-	-		-	-	+
23	9124		*05:03	*06:01	+	-		w	-	-
24		JBUSH	*03:01		-	-		-	-	-
25		IBW9	*02:02		-	-		-	-	-
26		WT49	*02:01		-	-		-	-	-
27		CH1007	*04:01	*05:01	-	-		+	-	-
28		BEL5GB	*02:02	*03:01	-	-		-	-	-
29	9050		*02:02			-		-	-	-
30	9021		*04:02			-		+	-	-
31		DUCAF	*02:01		-	-		-	-	-
32	9297		*03:01		+-	-		-	-	-
33		MT14B	*03:02		-	-	—	-	-	-
34	9104		*03:01		-	-		-	-	-
35		SSTO	*03:05			-		-	-	-
36		KT17	*03:02		+-	-		-	-	-
37		HHKB	*06:03		-	-		-	-	+
38	9099		*03:01	*02.04		-		-	-	-
39 40	9315		*02:01	*03:01	+-	-		-	-	-
		WHONP199	*02:02	*03:03	+-	-		-	-	-
41		H0301	*06:09		+-	-		•	-	+
42		TAB089	*06:01	+	+			w		
43		T7526	*03:03		-	-		-	-	-
44 45	9057		*05:03			-		-	-	-
		SHJO	*02:02		÷	-		-	-	-
46 47		SCHU	*06:02		+	+		-	-	-
			*03:01		-	-		-	-	-
48	9303	TER-ND	*05:01		-	- 1		-	-	-

Product Insert

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Lot No.: **96K**

Lot-specific information

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CELL LINE VALIDATION SHEET							
	DQA1*01:02						
				V	Vell		
				-	3	4	5
				 o	200962603	200962604	200962505
				Prod. No.:	962	962	962
				rod	80	ő	80
			-		Ñ	Ñ	Ñ
		VC cell line	-	<u>2A1*</u>			
1	9001		*01:01	****	+	-	-
2		LK707	*01:03	*03:03	-	+	-
3		E4181324	*01:03		-	+	-
4		GU373	*05:01		-	-	-
5		KAS011	*01:02	*00.04	+	+	-
6 7	9353		*01:03	*03:01	-	+	-
	9020		*05:01	-	-	-	-
8	9025		*03		-	-	-
9	9026		*03:01		-	-	-
10		LKT3	*03:03		-	-	-
11		PITOUT	*02:01		-	-	-
12	9052		*02:01	+	-	-	-
13		JESTHOM	*01:01		-	-	-
14		OLGA	*04:01		-	-	-
15	9075		*03:02		-	-	-
16		SWEIG007	*05:05	1001	-	-	-
17		CTM3953540	*01:03	*05:01	-	+	-
18		32367	*01:02	*03:03	+	+	-
19		BM16	*05:05		-	-	-
20		SLE005	*01:02		+	+	-
21		AMALA	*05:03		-	-	-
22		KOSE	*01:02	*01:04	+	+	-
23	9124		*01:03	*01:04	+	+	-
24		JBUSH	*05:05		-	-	-
25		IBW9	*02:01		-	-	-
26		WT49	*05:01		-	-	-
27		CH1007	*03:03	*01:05	+	-	-
28		BEL5GB	*02:01	*03:03	-	-	-
29		MOU	*02:01		-	-	-
30	9021		*04:01		-	-	-
31		DUCAF	*05:01		-	-	-
32	9297		*05:05	-	-	-	-
33		MT14B	*03:01		-	-	-
34		DHIF	*05:05		-	-	-
35		SSTO	*03:01		-	-	-
36		KT17	*03:01	<u> </u>	-	-	-
37		HHKB	*01:03		-	+	-
38	9099		*05:03		-	-	-
39	9315		*03:03	*05:01	-	-	-
40		WHONP199	*02:01	*03:02	-	-	-
41		H0301	*01:02		+	+	-
42		TAB089	*01:03	<u> </u>	-	+	-
43		T7526	*03:02		-	-	-
44		ТЕМ	*01:04		+	-	-
45	9239	SHJO	*02:01	*03:03	-	-	-
46		SCHU	*01:02		+	+	-
47		TUBO	*05:05		-	-	-
48	9303	TER-ND	*01:01		+	-	-

Lot-specific information

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CERTIFICATE OF ANALYSIS

Olerup SSP[®] DQB1*06:02,DQA1*01:02 - SSP

Product number:101.901-24 – including Taq polymeraseLot number:96KExpiry date:2013-July-01Number of tests:24Number of wells per test:8

Well specifications:

Well No.	Production No.
1	2009-626-01
2	2009-626-02
3	2009-626-03
4	2009-626-04
5	2009-626-05
6	2009-626-06
7	2010-815-07
8	2010-815-08

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 5 and 7 were available. The specificities of the primers in primer solutions 7 were tested by separately adding one additional 5'-primer, respectively, one additional 3'-primer. In primer solution 5 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 6 and 8 one 3'-primer was not possible to test, and in primer solution 7 two 5'-primers were not possible to test.

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

Date of approval: 2011-February-04

Approved by:

Quality Control, Supervisor

Lot-specific information

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Declaration of Conformity

Product name: Product number: Lot number:	<i>Olerup</i> SSP [®] DQB1*06:02,DQA1*01:02 101.901-24 96K
Intended use:	DQB1*06:02,DQA1*01:02 histocompatibility testing
Manufacturer:	<i>Olerup</i> SSP AB Hasselstigen 1 SE-133 33 Saltsjöbaden, Sweden <i>Phone:</i> +46-8-717 88 27 <i>Fax:</i> +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, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

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

Saltsjöbaden, Sweden 2011-February-04

Olle Olerup Managing Director

Lot-specific information

www.olerup-ssp.com

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