

Olerup SSP[®] DQB1*06

Product number:	101.212-24/04 – including <i>Taq</i> pol.
Lot number:	40K
Expiry date:	2012-September-01
Number of tests:	24 test – Product No. 101.212-24 4 tests – Product No. 101.212-04
Number of wells per test:	30
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. 40K.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP[®] DQB1*06 LOT

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

One well has been added to the DQB1*06 kit,
well **30**.

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

Well	5'-primer	3'-primer	rationale
11	Added	-	Primer added for increased yield of specific PCR product.
19	Added	Added	Primer pair added for the DQB1*06:36 allele.
20	Added	-	Primer added for the DQB1*06:37 allele.
22	Added	Added	Primer pair added for the DQB1*06:38 allele.
26	Added	-	Primer added for the DQB1*06:35 allele.
27	Added	-	Primer added for the DQB1*06:40 allele.
30	New	New	New primer pair for the DQB1*06:39 allele.

Change in revision R01 compared to R00:

1. The DQB1*06:28 allele is weakly amplified by primer mix 26.

Change in revision R02 compared to R01:

1. A footnote (no. 13), concerning primers in incompletely sequenced regions, has been added to the specificity table.

Change in revision R03 compared to R02:

1. The DQB1*06:20 allele is weakly amplified by primer mix 10.

PRODUCT DESCRIPTION

DQB1*06 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DQB1*06:01 to DQB1*06:40 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.

STRIP LAYOUT

Each test consists of 30 PCR reactions in a 32 well cut PCR plate. Wells 31 and 32 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	empty	empty

The 32 well cut PCR plate is marked with ‘DQB1*06’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘40K’.

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 32 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 the DQB1*06 alleles will be amplified by the DQB1*06 subtyping kit, except that the DQB1*03:23 allele will be amplified by primer mix 5, and DQB1*04:01:01-04:03:02 alleles will be amplified by primer mix 24. Thus, the interpretation of DQB1*06 subtypings is only influenced by a few non-DQB1*06 alleles and not by other groups of DQB1 alleles or the DQB2 and DQB3 genes.

UNIQUELY IDENTIFIED ALLELES

All the DQB1*06 alleles, i.e. **DQB1*06:01 to DQB1*06:40**, recognized by the HLA Nomenclature Committee in July 2010¹ will give rise to unique amplification patterns by the primers in the DQB1*06 subtyping kit.

The DQB1*06 subtyping kit cannot distinguish the DQB1*06:01:01-06:01:05 alleles, the DQB1*06:02:01-06:02:02 alleles, the DQB1*06:03:01-06:03:02 alleles or the DQB1*06:04:01 and 06:04:03 alleles.

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

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Lot-specific information

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RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 52 alleles generate 45 amplification patterns that can be combined in 1035 homozygous and heterozygous combinations. 236 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.

+++++--	-----	-----+	+++++	*06:01:01, *06:28 = *06:03:01, *06:35 = *06:28, *06:35
+++++++	+++++	-----	+++++	*06:04:01, *06:35 = *06:35, *06:39
+++++++	-----	-----	+++++	*06:01:01, *06:35 = *06:35, *06:35
+++++--	-----	-----+	+++++	*06:20, *06:40 = *06:31, *06:33
+++++--	-----	-----+	+++++	*06:02:01, *06:31 = *06:03:01, *06:20 = *06:20, *06:31
+++++--	-----	-----+	+++++	*06:11:01, *06:14:01 = *06:11:02, *06:14:01 = *06:11:02, *06:14:02
+++++--	-----	-----+	+++++	*06:02:01, *06:26N = *06:11:02, *06:37 = *06:26N, *06:37
+++++--	-----	-----+	+++++	*06:02:01, *06:40 = *06:03:01, *06:33 = *06:33, *06:40
+++++--	-----	-----+	+++++	*06:02:01, *06:30 = *06:24, *06:30
+++++--	-----	-----+	+++++	*06:02:01, *06:14:01 = *06:14:01, *06:29
+++++++	+++++	-----	+++++	*06:04:01, *06:20 = *06:20, *06:39
+++++++	+++++	-----	+++++	*06:04:01, *06:16 = *06:16, *06:39
+++++++	+++++	-----	+++++	*06:04:01, *06:24 = *06:24, *06:39
+++++++	+++++	-----	+++++	*06:04:01, *06:37 = *06:37, *06:39
+++++++	+++++	-----	+++++	*06:04:01, *06:33 = *06:33, *06:39
+++++++	+++++	-----	+++++	*06:02:01, *06:04:01 = *06:02:01, *06:39
+++++++	+++++	-----	+++++	*06:04:01, *06:19 = *06:19, *06:39
+++++++	+++++	-----	+++++	*06:02:01, *06:17 = *06:17, *06:24
+++++++	+++++	-----	+++++	*06:02:01, *06:06 = *06:06, *06:20
+++++++	+++++	-----	+++++	*06:02:01, *06:05:01 = *06:05:01, *06:20 = *06:09, *06:20
+++++++	+++++	-----	+++++	*06:02:01, *06:05:02 = *06:05:02, *06:20
+++++++	+++++	-----	+++++	*06:02:01, *06:20 = *06:20, *06:20
+++++++	+++++	-----	+++++	*06:02:01, *06:14:02 = *06:14:02, *06:29
+++++++	+++++	-----	+++++	*06:02:01, *06:16 = *06:16, *06:16
+++++++	+++++	-----	+++++	*06:02:01, *06:24 = *06:24, *06:24
+++++++	+++++	-----	+++++	*06:02:01, *06:37 = *06:37, *06:37
+++++++	+++++	-----	+++++	*06:02:01, *06:33 = *06:33, *06:33
+++++++	+++++	-----	+++++	*06:04:01, *06:11:02 = *06:04:02, *06:11:02 = *06:11:02, *06:39
+++++++	+++++	-----	+++++	*06:03:01, *06:06 = *06:06, *06:31
+++++++	+++++	-----	+++++	*06:03:01, *06:05:02 = *06:05:02, *06:31
+++++++	+++++	-----	+++++	*06:08:01, *06:11:02 = *06:08:02, *06:11:02
+++++++	+++++	-----	+++++	*06:11:01, *06:31 = *06:11:02, *06:31
+++++++	+++++	-----	+++++	*06:11:01, *06:26N = *06:11:02, *06:26N
+++++++	+++++	-----	+++++	*06:11:01, *06:28 = *06:11:02, *06:28
+++++++	+++++	-----	+++++	*06:11:01, *06:40 = *06:11:02, *06:40
+++++++	+++++	-----	+++++	*06:03:01, *06:11:01 = *06:03:01, *06:11:02
+++++++	+++++	-----	+++++	*06:11:01, *06:11:02 = *06:11:02, *06:11:02
+++++++	+++++	-----	+++++	*06:08:01, *06:11:01 = *06:08:02, *06:11:01
+++++++	+++++	-----	+++++	*06:04:01, *06:11:01 = *06:04:02, *06:11:01 = *06:04:02, *06:18 = *06:11:01, *06:39
+++++++	+++++	-----	+++++	*06:06, *06:25 = *06:06, *06:36
+++++++	+++++	-----	+++++	*06:04:01, *06:06 = *06:06, *06:39
+++++++	+++++	-----	+++++	*06:04:01, *06:05:02 = *06:05:02, *06:39
+++++++	+++++	-----	+++++	*06:04:01, *06:10 = *06:10, *06:36 = *06:10, *06:39
+++++++	+++++	-----	+++++	*06:04:01, *06:13 = *06:13, *06:39
+++++++	+++++	-----	+++++	*06:04:01, *06:29 = *06:29, *06:39
+++++++	+++++	-----	+++++	*06:18, *06:25 = *06:18, *06:36
+++++++	+++++	-----	+++++	*06:04:01, *06:18 = *06:18, *06:39
+++++++	+++++	-----	+++++	*06:05:01, *06:06 = *06:05:02, *06:06 = *06:06, *06:06 = *06:06, *06:09 = *06:06, *06:18
+++++++	+++++	-----	+++++	*06:05:01, *06:05:02 = *06:05:01, *06:18 = *06:05:02, *06:09 = *06:05:02, *06:18
+++++++	+++++	-----	+++++	*06:14:01, *06:31 = *06:14:02, *06:31
+++++++	+++++	-----	+++++	*06:14:01, *06:26N = *06:14:02, *06:26N



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-----+-----+-----+ +-----+	*06:14:01, *06:28 = *06:14:02, *06:28
-----+-----+-----+ +-----+	*06:14:01, *06:40 = *06:14:02, *06:40
-----+-----+-----+ +-----+	*06:03:01, *06:14:01 = *06:03:01, *06:14:02
-----+-----+-----+ +-----+	*06:04:01, *06:14:01 = *06:14:01, *06:39
-----+-----+-----+ +-----+	*06:14:01, *06:14:01 = *06:14:01, *06:14:02
-----+-----+-----+ +-----+	*06:04:01, *06:15 = *06:15, *06:38 = *06:15, *06:39
-----+-----+-----+ +-----+	*06:04:01, *06:14:02 = *06:14:02, *06:39
-----+-----+-----+ +-----+	*06:04:01, *06:23 = *06:23, *06:39
-----+-----+-----+ +-----+	*06:04:01, *06:31 = *06:04:02, *06:31 = *06:31, *06:39
-----+-----+-----+ +-----+	*06:04:01, *06:26N = *06:04:02, *06:26N = *06:26N, *06:39
-----+-----+-----+ +-----+	*06:03:01, *06:38 = *06:07, *06:08:01 = *06:07, *06:08:02
-----+-----+-----+ +-----+	*06:04:01, *06:28 = *06:04:02, *06:28 = *06:28, *06:39
-----+-----+-----+ +-----+	*06:04:01, *06:40 = *06:04:02, *06:40 = *06:39, *06:40
-----+-----+-----+ +-----+	*06:03:01, *06:04:01 = *06:03:01, *06:04:02 = *06:03:01, *06:39
-----+-----+-----+ +-----+	*06:03:01, *06:17 = *06:21, *06:30
-----+-----+-----+ +-----+	*06:03:01, *06:05:01 = *06:05:01, *06:31 = *06:09, *06:31
-----+-----+-----+ +-----+	*06:03:01, *06:27 = *06:08:01, *06:32 = *06:08:02, *06:32
-----+-----+-----+ +-----+	*06:08:01, *06:31 = *06:08:02, *06:31
-----+-----+-----+ +-----+	*06:08:01, *06:30 = *06:08:02, *06:30
-----+-----+-----+ +-----+	*06:08:01, *06:26N = *06:08:02, *06:26N
-----+-----+-----+ +-----+	*06:08:01, *06:28 = *06:08:02, *06:28
-----+-----+-----+ +-----+	*06:08:01, *06:40 = *06:08:02, *06:40
-----+-----+-----+ +-----+	*06:03:01, *06:08:01 = *06:03:01, *06:08:02
-----+-----+-----+ +-----+	*06:03:01, *06:31 = *06:31, *06:31
-----+-----+-----+ +-----+	*06:03:01, *06:28 = *06:28, *06:28
-----+-----+-----+ +-----+	*06:03:01, *06:40 = *06:40, *06:40
-----+-----+-----+ +-----+	*06:04:01, *06:30 = *06:04:02, *06:30 = *06:30, *06:39
-----+-----+-----+ +-----+	*06:07, *06:27 = *06:32, *06:38
-----+-----+-----+ +-----+	*06:04:01, *06:07 = *06:04:02, *06:07 = *06:07, *06:38 = *06:07, *06:39
-----+-----+-----+ +-----+	*06:04:01, *06:32 = *06:04:02, *06:32 = *06:32, *06:39
-----+-----+-----+ +-----+	*06:08:01, *06:25 = *06:08:01, *06:36
-----+-----+-----+ +-----+	*06:04:01, *06:08:01 = *06:04:02, *06:08:01 = *06:04:02, *06:08:02 = *06:08:01, *06:39
-----+-----+-----+ +-----+	*06:08:01, *06:08:01 = *06:08:01, *06:08:02
-----+-----+-----+ +-----+	*06:04:01, *06:04:02 = *06:04:02, *06:04:02
-----+-----+-----+ +-----+	*06:08:02, *06:25 = *06:08:02, *06:36
-----+-----+-----+ +-----+	*06:21, *06:25 = *06:21, *06:36
-----+-----+-----+ +-----+	*06:04:01, *06:08:02 = *06:08:02, *06:39
-----+-----+-----+ +-----+	*06:04:01, *06:21 = *06:21, *06:39
-----+-----+-----+ +-----+	*06:05:01, *06:25 = *06:05:01, *06:36
-----+-----+-----+ +-----+	*06:04:01, *06:05:01 = *06:05:01, *06:39
-----+-----+-----+ +-----+	*06:04:01, *06:22 = *06:22, *06:39
-----+-----+-----+ +-----+	*06:17, *06:25 = *06:17, *06:36
-----+-----+-----+ +-----+	*06:04:01, *06:17 = *06:17, *06:39
-----+-----+-----+ +-----+	*06:25, *06:27 = *06:27, *06:36
-----+-----+-----+ +-----+	*06:04:01, *06:25 = *06:25, *06:36 = *06:25, *06:39 = *06:36, *06:39
-----+-----+-----+ +-----+	*06:04:01, *06:36 = *06:36, *06:36
-----+-----+-----+ +-----+	*06:04:01, *06:38 = *06:38, *06:38
-----+-----+-----+ +-----+	*06:04:01, *06:27 = *06:27, *06:39
-----+-----+-----+ +-----+	*06:04:01, *06:34 = *06:34, *06:34
-----+-----+-----+ +-----+	*06:04:01, *06:39 = *06:39, *06:39
-----+-----+-----+ +-----+	*06:05:01, *06:05:01 = *06:05:01, *06:09

*06:01:01 = *06:01:01-06:01:05
*06:02:01 = *06:02:01-06:02:02
*06:03:01 = *06:03:01-06:03:02
*06:04:01 = *06:04:01 and 06:04:03



SPECIFICITY TABLE

DQB1*06 SSP subtyping

Specificities and sizes of the PCR products of the 30 primer mixes used for DQB1*06 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DQB1*06 alleles ¹³	Amplified non-DQB1*06 alleles ³
1	220 bp	515 bp	*06:01:01-06:01:05, 06:35	
2	210 bp	430 bp	*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	
3	185 bp	430 bp	*06:02:01-06:02:02, 06:14:01-06:16, 06:19-06:20, 06:23-06:24, 06:33, 06:37	
4	130 bp	430 bp	*06:03:01-06:03:02, 06:07, 06:11:02, 06:14:01, 06:26N, 06:28, 06:30-06:32, 06:40	
5	160 bp	430 bp	*06:03:01-06:03:02, 06:04:02, 06:07-06:08:01, 06:11:01-06:11:02, 06:26N, 06:28, 06:30-06:32, 06:40	*03:23
6	170 bp	515 bp	*06:03:01-06:03:02, 06:08:01-06:08:02, 06:11:02-06:12, 06:14:01, 06:21, 06:26N, 06:28, 06:31, 06:40	
7	210 bp	515 bp	*06:04:01-06:04:03, 06:07, 06:17, 06:21, 06:25, 06:34, 06:36, 06:38-06:39	
8	170 bp	430 bp	*06:04:01-06:07, 06:09, 06:18, 06:25, 06:27, 06:32, 06:34, 06:36, 06:38-06:39	
9⁵	130 bp	430 bp	*06:04:01-06:05:01, 06:06, 06:08:01-06:09, 06:12, 06:17-06:18, 06:21, 06:27, 06:34, 06:36, 06:38-06:39	
10⁶	260 bp	515 bp	*06:05:01, 06:05:02 [?] -06:06 [?] , 06:20 ^w , 06:31	
11	210 bp	430 bp	*06:05:01, 06:05:02 [?] -06:06 [?] , 06:09, 06:12, 06:15, 06:22	
12	180 bp	430 bp	*06:06	

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13	185 bp	430 bp	*06:10	
14	130 bp	430 bp	*06:13, 06:22	
15^{4,8}	100 bp, 185 bp	430 bp	*06:14:01-06:14:02, 06:29	
16	195 bp	430 bp	*06:16	
17⁴	110 bp	430 bp	*06:23	
18⁷	175 bp	430 bp	*06:17, 06:24, 06:30	
19	135 bp	430 bp	*06:10, 06:25, 06:36	
20^{4,9}	110 bp, 215 bp	515 bp	*06:26N, 06:37	
21	160 bp	430 bp	*06:02:01-06:02:02, 06:10, 06:13-06:16, 06:20, 06:23- 06:24, 06:29, 06:33, 06:37	
22¹⁰	130 bp, 195 bp	515 bp	*06:07, 06:15, 06:38	
23	160 bp	515 bp	*06:03:01-06:03:02, 06:08:01-06:08:02, 06:14:01- 06:14:02, 06:21, 06:28, 06:31, 06:40	
24⁵	155 bp	430 bp	*06:19	*04:01:01- 04:03:02
25	210 bp	430 bp	*06:03:01-06:03:02, 06:08:01-06:08:02, 06:14:01- 06:14:02, 06:27-06:28, 06:30- 06:32, 06:40	
26¹¹	165 bp, 190 bp	430 bp	*06:28 ^w , 06:35	
27¹²	205 bp, 265 bp	430 bp	*06:33, 06:40	
28	300 bp	430 bp	*06:34	
29⁴	90 bp	430 bp	*06:04:01-06:05:01, 06:05:02 [?] -06:06 [?] , 06:07, 06:09, 06:15, 06:22, 06:25, 06:34, 06:36, 06:38-06:39	
30^{4,5}	115 bp	430 bp	*06:02:01-06:03:02, 06:05:01 [?] -06:08:02 [?] , 06:10 [?] - 06:11:02 [?] , 06:13 [?] -06:33 [?] , 06:35 [?] , 06:37 [?] , 06:39, 06:40 [?]	

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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 DQB1*06 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. 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 subtyping.

In addition, wells number 6, 7, 10, 20, 22 and 23 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.

³Due to the sharing of sequence motif between DQB1 alleles, the DQB1*03:23 alleles will be amplified by primer mix 5, and the DQB1*04:01:01-04:03:02 alleles will be amplified by primer mix 24.

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

⁵Primer mix 9, 24 and 30 may yield less specific PCR product than the other DQB1*06 primer mixes, most pronounced in primer mixes 24 and 30.

⁶The nucleotide sequence of codon 14 of the DQB1*06:05:02 allele is not yet known. If codon 14 is CTg, then the DQB1*06:05:02 allele will retain its name and will be amplified by the primer pair in well No. 10. If the sequence of codon 14 is ATg, then DQB1*06:05:02 will be renamed to DQB1*06:09:02 (Steven Marsh personal communication), and will not be amplified by the primer pair in well No. 10.

⁷Primer mix 18 may give rise to primer dimer formation.

⁸Primer mix 15: Specific PCR fragment of 100 bp in the DQB1*06:14:01 and *06:14:02 alleles. Specific PCR fragment of 185 bp in the DQB1*06:29 allele.

⁹Primer mix 20: Specific PCR fragment of 110 bp in the DQB1*06:37 allele. Specific PCR fragment of 215 bp in the DQB1*06:26N allele.

¹⁰Primer mix 22: Specific PCR fragment of 130 bp in the DQB1*06:07 and 06:15 alleles. Specific PCR fragment of 185 bp in the DQB1*06:38 allele.

¹¹Primer mix 26: Specific PCR fragment of 165 bp in the DQB1*06:35 allele. Specific PCR fragment of 190 bp in the DQB1*06:28^w allele.

¹²Primer mix 27: Specific PCR fragment of 205 bp in the DQB1*06:40 allele. Specific PCR fragment of 265 bp in the DQB1*06:33 allele.

¹³For several DQB1 alleles only second 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 of DQB1 alleles are conserved within allelic groups.

'w', might be weakly amplified.

"?", nucleotide sequence information is not available for the primer matching sequence.

INTERPRETATION TABLE

DQB1*06 SSP subtyping

Amplification patterns of the DQB1*06:01 to 06:40 alleles

	Well ⁴															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length of spec.	220	210	185	130	160	170	210	170	130	260	210	180	185	130	100	195
PCR product(s)															185	
Length of int.	515	430	430	430	430	515	515	430	430	515	430	430	430	430	430	430
pos. control ¹																
5'-primer(s) ²	26 (173)	30 (184)	9 (122)	27 (177)	9 (122)	27 (177)	30 (184)	27 (177)	27 (177)	14 (136)	30 (184)	27 (177)	9 (122)	27 (177)	9 (122)	9 (122)
	5'-TTA ^{3'}	5'-gAT ^{3'}	5'-gTT ^{3'}	5'-gTA ^{3'}	5'-gTA ^{3'}	5'-gTA ^{3'}	5'-gAC ^{3'}	5'-gTA ^{3'}	5'-gTA ^{3'}	5'-gCC ^{3'}	5'-gAT ^{3'}	5'-gTA ^{3'}	5'-gTT ^{3'}	5'-gTg ^{3'}	5'-gTT ^{3'}	5'-gTT ^{3'}
		30 (184)														
		5'-gAT ^{3'}														
3'-primer(s) ³	86 (353)	86 (353)	57 (266)	57 (266)	48 (240)	70 (304)	86 (353)	70 (304)	57 (266)	86 (353)	86 (353)	74 (317)	57 (265)	57 (266)	27 (177)	60 (274)
	5'-ACg ^{3'}	5'-ACg ^{3'}	5'-CAT ^{3'}	5'-CAT ^{3'}	5'-gCg ^{3'}	5'-CCC ^{3'}	5'-ACC ^{3'}	5'-CCT ^{3'}	5'-CAA ^{3'}	5'-ACC ^{3'}	5'-ACC ^{3'}	5'-CCg ^{3'}	5'-gCT ^{3'}	5'-CAA ^{3'}	5'-gTT ^{3'}	5'-gTT ^{3'}
								74 (317)		87 (356)					30 (184)	
								5'-CCg ^{3'}		5'-gga ^{3'}					5'-gTg ^{3'}	
															57 (266)	
															5'-Cgg ^{3'}	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
DQB1 allele																
*06:01:01-06:01:05	1	2														
*06:02:01-06:02:02		2	3													
*06:03:01-06:03:02				4	5	6										
*06:04:01, 06:04:03							7	8	9							
*06:04:02					5		7	8	9							
*06:05:01								8	9	10	11					
*06:05:02		?						8		?	?					
*06:06		?						8	9	?	?	12				
*06:07				4	5		7	8								
*06:08:01					5	6			9							
*06:08:02						6			9							
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

INTERPRETATION TABLE														
DQB1*06 SSP subtyping														
Amplification patterns of the DQB1*06:01 to 06:40 alleles														
Well ⁴														
17	18	19	20	21	22	23	24	25	26	27	28	29	30	
110	175	135	110	160	130	160	155	210	165	205	300	90	115	Length of spec. PCR product(s)
			215		195				190	265				Length of int. pos. control ¹
430	430	430	515	430	515	515	430	430	430	430	430	430	430	5'-primer(s) ²
26 (173)	26 (173)	26 (173)	29 (181)	9 (122)	57 (266)	30 (184)	9 (122)	30 (184)	38 (209)	11 (129)	102 (400)	70 (304)	130 (485)	
5'-ggg ^{3'}	5'-TCT ^{3'}	5'-TCT ^{3'}	5'-CCT ^{3'}	5'-gTT ^{3'}	5'-TgA ^{3'}	5'-gAC ^{3'}	5'-gTT ^{3'}	5'-gAC ^{3'}	5'-CgT ^{3'}	5'-TTA ^{3'}	5'-TCT ^{3'}	5'-AgA ^{3'}	5'-CCg ^{3'}	
		154 (558)	62 (282)		133 (494)				45 (230)	31 (187)				
		5'-ACT ^{3'}	5'-AAG ^{3'}		5'-TCA ^{3'}				5'-ggA ^{3'}	5'-ACg ^{3'}				
48 (240)	71 (307)	57 (265)	86 (353)	48 (240)	86 (353)	70 (304)	47 (237)	86 (353)	87 (356)	86 (353)	189 (661)	86 (353)	154 (558)	3'-primer(s) ³
5'-gCg ^{3'}	5'-ggC ^{3'}	5'-gCT ^{3'}	5'-ACg ^{3'}	5'-gCg ^{3'}	5'-ACC ^{3'}	5'-CCC ^{3'}	5'-CgA ^{3'}	5'-ACg ^{3'}	5'-ggA ^{3'}	5'-ACg ^{3'}	5'-CCA ^{3'}	5'-ACC ^{3'}	5'-AAA ^{3'}	
		186 (653)			185 (650)									
		5'-CCg ^{3'}			5'-Cgg ^{3'}									
17	18	19	20	21	22	23	24	25	26	27	28	29	30	Well No.
														DQB1 allele
														*06:01:01-06:01:05
				21									30	*06:02:01-06:02:02
						23		25					30	*06:03:01-06:03:02
												29		*06:04:01, 06:04:03
												29		*06:04:02
												29	?	*06:05:01
												?	?	*06:05:02
												?	?	*06:06
					22							29	?	*06:07
						23		25					?	*06:08:01
						23		25					?	*06:08:02
17	18	19	20	21	22	23	24	25	26	27	28	29	30	Well No.

Lot No.: **40K**

Lot-specific information

www.olerup-ssp.com

Length of spec.	220	210	185	130	160	170	210	170	130	260	210	180	185	130	100	195
PCR product(s)															185	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*06:09								8	9		11					
*06:10		2											13			
*06:11:01		2			5											
*06:11:02		2		4	5	6										
*06:12						6			9		11					
*06:13		2												14		
*06:14:01			3	4		6									15	
*06:14:02			3												15	
*06:15			3								11					
*06:16		2	3													16
*06:17							7		9							
*06:18		2						8	9							
*06:19		2	3													
*06:20		2	3							w						
*06:21						6	7		9							
*06:22											11			14		
*06:23			3													
*06:24		2	3													
*06:25							7	8								
*06:26N				4	5	6										
*06:27								8	9							
*06:28				4	5	6										
*06:29		2													15	
*06:30				4	5											
*06:31				4	5	6				10						
*06:32				4	5			8								
*06:33		2	3													
*06:34							7	8	9							
*06:35	1	2														
*06:36							7	8	9							
*06:37		2	3													
*06:38							7	8	9							
*06:39							7	8	9							
*06:40				4	5	6										
*03:23					5											
*04:01:01-04:03:02																
DQB1 allele																
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Lot No.: **40K**

Lot-specific information

www.olerup-ssp.com

110	175	135	110	160	130	160	155	210	165	205	300	90	115	Length of spec. PCR product(s)
			215		195				190	265				Well No.
17	18	19	20	21	22	23	24	25	26	27	28	29	30	
												29		*06:09
		19		21									?	*06:10
													?	*06:11:01
													?	*06:11:02
														*06:12
				21									?	*06:13
				21		23		25					?	*06:14:01
				21		23		25					?	*06:14:02
				21	22							29	?	*06:15
				21									?	*06:16
	18												?	*06:17
													?	*06:18
							24						?	*06:19
				21									?	*06:20
						23							?	*06:21
												29	?	*06:22
17				21									?	*06:23
	18			21									?	*06:24
		19										29	?	*06:25
			20										?	*06:26N
								25					?	*06:27
						23		25	w				?	*06:28
				21									?	*06:29
	18							25					?	*06:30
						23		25					?	*06:31
								25					?	*06:32
				21						27			?	*06:33
											28	29		*06:34
								26					?	*06:35
		19										29		*06:36
			20	21									?	*06:37
					22							29		*06:38
												29	30	*06:39
						23		25		27			?	*06:40
							24							*03:23
														*04:01:01-04:03:02
														DQB1 allele
17	18	19	20	21	22	23	24	25	26	27	28	29	30	Well No.

Lot No.: **40K**

Lot-specific information

www.olerup-ssp.com

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 subtyping.

In addition, wells number 6, 7, 10, 20, 22 and 23 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

³The codon, and in parenthesis the nucleotide, in the 2nd or 3rd exons, 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 or 3rd exons, 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.

⁴Primer mix 15: Specific PCR fragment of 100 bp in the DQB1*06:14:01 and *06:14:02 alleles. Specific PCR fragment of 185 bp in the DQB1*06:29 allele.

Primer mix 20: Specific PCR fragment of 110 bp in the DQB1*06:37 allele. Specific PCR fragment of 215 bp in the DQB1*06:26N allele.

Primer mix 22: Specific PCR fragment of 130 bp in the DQB1*06:07 and 06:15 alleles. Specific PCR fragment of 185 bp in the DQB1*06:38 allele.

Primer mix 26: Specific PCR fragment of 165 bp in the DQB1*06:35 allele. Specific PCR fragment of 190 bp in the DQB1*06:28^w allele.

Primer mix 27: Specific PCR fragment of 205 bp in the DQB1*06:40 allele. Specific PCR fragment of 265 bp in the DQB1*06:33 allele.

'w', might be weakly amplified.

"?", nucleotide sequence information is not available for the primer matching sequence.

CELL LINE VALIDATION SHEET																				
DQB1*06 SSP subtyping kit																				
				Prod. No.	Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	IHWC cell line	DQB1			201075701	201075702	201075703	201075704	201075705	201075706	201075707	201075708	201075709	201075710	201075711	201075712	201075713	201075714	201075715	201075716
1	9001 SA	*05:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707	*06:01	*02:02		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324	*06:01			+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*05:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM	*03:02	*06:01		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025 DEU	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*03:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3	*04:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*03:03			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM	*05:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*04:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*03:03			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540	*02:01	*06:03		-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*06:02	*02:02		-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*06:04			-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
21	9064 AMALA	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*05:03	*06:04		-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
23	9124 IHL	*05:03	*06:01		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*04:01	*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB	*02:02	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*04:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*03:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*03:05			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*03:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB	*06:03			-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML	*02:01	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*02:02	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*06:09			-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-
42	9066 TAB089	*06:01			+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*03:03			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*05:03			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU	*06:02			-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND	*05:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CELL LINE VALIDATION SHEET																		
DQB1*06 SSP subtyping kit																		
				Prod. No.	Well													
					17	18	19	20	21	22	23	24	25	26	27	28	29	30
					201075717	201075718	201075719	201075720	201075721	201075722	201075723	201075724	201075725	201075726	201075727	201075728	201075729	201075730
	IHWC cell line		DQB1															
1	9001 SA		*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707		*06:01	*02:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324		*06:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373		*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011		*05:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM		*03:02	*06:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL		*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025 DEU		*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR		*03:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3		*04:01		-	-	-	-	-	-	-	+	-	-	-	-	-	-
11	9051 PITOUT		*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB		*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM		*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA		*04:02		-	-	-	-	-	-	-	+	-	-	-	-	-	-
15	9075 DKB		*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007		*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540		*02:01	*06:03	-	-	-	-	-	-	+	-	+	-	-	-	-	+
18	9257 32367		*06:02	*02:02	-	-	-	-	+	-	-	-	-	-	-	-	-	+
19	9038 BM16		*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005		*06:04		-	-	-	-	-	-	-	-	-	-	-	-	-	+
21	9064 AMALA		*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE		*05:03	*06:04	-	-	-	-	-	-	-	-	-	-	-	-	-	+
23	9124 IHL		*05:03	*06:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH		*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9		*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49		*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007		*04:01	*05:01	-	-	-	-	-	-	-	+	-	-	-	-	-	-
28	9320 BEL5GB		*02:02	*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU		*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH		*04:02		-	-	-	-	-	-	-	+	-	-	-	-	-	-
31	9019 DUCAF		*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG		*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B		*03:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF		*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO		*03:05		-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17		*03:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB		*06:03		-	-	-	-	-	-	+	-	+	-	-	-	-	+
38	9099 LZL		*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML		*02:01	*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199		*02:02	*03:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301		*06:09		-	-	-	-	-	-	-	-	-	-	-	-	-	+
42	9066 TAB089		*06:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526		*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM		*05:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO		*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU		*06:02		-	-	-	-	+	-	-	-	-	-	-	-	-	+
47	9045 TUBO		*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND		*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-



CERTIFICATE OF ANALYSIS

OLERUP SSP® DQB1*06 SSP

Product number: 101.212-24/04 – including *Taq* pol.
Lot number: 40K
Expiry date: 2012-September-01
Number of tests: 24 test – Product No. 101.212-24
4 tests – Product No. 101.212-04
Number of wells per test: 30

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2010-757-01	11	2010-757-11	21	2010-757-21
2	2010-757-02	12	2010-757-12	22	2010-757-22
3	2010-757-03	13	2010-757-13	23	2010-757-23
4	2010-757-04	14	2010-757-14	24	2010-757-24
5	2010-757-05	15	2010-757-15	25	2010-757-25
6	2010-757-06	16	2010-757-16	26	2010-757-26
7	2010-757-07	17	2010-757-17	27	2010-757-27
8	2010-757-08	18	2010-757-18	28	2010-757-28
9	2010-757-09	19	2010-757-19	29	2010-757-29
10	2010-757-10	20	2010-757-20	30	2010-757-30

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

No DNAs carrying the alleles to be amplified by primer solutions 10, 12 to 20, 22 and 26 to 28 were available. The specificities of the primers in primer solutions 10, 12 to 15, 17, 19, 22 and 26 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 16, 18 and 28 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 20 and 27 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solution 19, one 3'-primer was not possible to test, and in primer solution 22 one 5'-primer was not possible to test.

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

Date of approval: 2011-October-06

Approved by:

Quality Control, Supervisor

Lot No.: **40K**

Lot-specific information

www.olerup-ssp.com

Declaration of Conformity

Product name: *Olerup* SSP™ DQB1*06
Product number: 101.212-24/04
Lot number: 40K

Intended use: DQB1*06 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
2011-October-06

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

Lot No.: **40K**

Lot-specific information

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