Olerup SSP™ DQB1*03

Product number:	**101.214-24/06 – licensed for PCR** **101.214-24u/06u – not licensed for PCR**
Lot number:	**Y07**
Expiry date:	**2009-July-01**
Number of tests:	**24 test – Product No. 101.214-24** **6 tests – Product No. 101.214-06**
Number of tubes per test:	**16**
Storage - pre-aliquoted primers:	**dark at -20°C**
- PCR Master Mix:	**-20°C**

This Product Description is only valid for Lot No. Y07.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP™ DQB1*03 LOT

The DQB1*03 specificity and interpretation tables have been updated for the HLA-DQB1 alleles described since the previous *Olerup* SSP™ DQB1*03 lot was made **(Lot No. V55)**.

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

Tube	5'-primer	3'-primer	rationale
4	Added	Added	Primer pair added for the DQB1*0319 allele.
6	Added	-	Primer added for the DQB1*0320 allele.
14	-	Added	Primer added for the DQB1*030103 allele.

Changes in revision R01 compared to R00:

1. DQB1*0401 and 0402 added to alleles amplified by primer mix 4 in the Specificity Table.

PRODUCT DESCRIPTION

DQB1*03 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DQB1*0301 to DQB1*0320 alleles.
The primer solutions are pre-aliquoted into 0.2 ml PCR tubes. Each tube in the set contains a dried primer solution consisting of a specific primer mix, i.e. allele- and group-specific primers as well as a ***control primer pair*** matching non-allelic sequences.
PCR Master Mix complete with Taq, *Taq* polymerase, nucleotides, buffer, glycerol and cresol red, as well as PCR lids are included in the licensed kit.
PCR Master Mix without Taq, nucleotides, buffer, glycerol and cresol red, as well as PCR lids are included in the unlicensed kit.
16 PCR reactions with a reaction volume of 10 µl are performed per sample.
Note: The pellets in the tubes may vary in form and colour. This does not affect the performance of the product.

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 16 PCR reactions in an 16 well 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 'DQB1*03 Y07'.
Well No. 1 is marked with '1'.
The PCR plates are covered with a PCR-compatible foil.
Please note: When removing each 16 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*03 alleles will be amplified by the DQB1*03 subtyping kit, except that non-DQB1*03 alleles, DQB1*02, DQB1*04, DQB1*0601, DQB1*060302 and DQB1*0629, will be amplified by primer mixes 3, 4, 9 and 11. Thus, the interpretation of DQB1*03 subtypings is only influenced these DQB1 alleles and not by other groups of DQB1 alleles or the DQB2 and DQB3 genes.

UNIQUELY IDENTIFIED ALLELES

All the DQB1*03 alleles, i.e. **DQB1*0301 to DQB1*0320**, recognized by the HLA Nomenclature Committee in July 2007[1] will give rise to unique amplification patterns by the primers in the DQB1*03 subtyping kit.
The DQB1*03 subtyping kit cannot distinguish the DQB1*030101 and DQB1*030102 alleles or the DQB1*030201 to DQB1*030204 alleles or the DQB1*030302 and DQB1*030303 alleles or the DQB1*030501 to DQB1*030504 alleles.

[1]***Nomenclature for factors of the HLA system, 1998***. *Tissue Antigens* 1999: **53**: 407-446 and DQB1 alleles listed on the IMGT/HLA web page 2007-July-09, release 2.18.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 20 DQB1*03 alleles give rise to 21 different amplification patterns that can be combined in 231 homozygous and heterozygous combinations. 128 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products generated by primer mixes 4, 6, 8 and 10 were not considered in these calculations.

```
+++---+ -+-+-+++    0301,0318 = 0307,0309 = 0309,0318 =
                     0318,0319
+++---+ -+-+--+-    0311,0316 = 0316,0318
+++---+ ---+-+++    0301,0307 = 0307,0319
+++---+ ---+--+-    0302,0316 = 0307,0316
+++--- +--+-+++    0301,0308 = 0308,0319
+++--- -+-+-+++    0301,0311 = 0302,0309 = 0309,0311 =
                     0311,0319
+++--- ---+-+++    0301,0302 = 0302,0319
++-++-- --++-+++    0304,0312 = 0314,0319
++-++-- ---+-+++    0303,0304 = 0304,0319
++-+-+-- -+-+-+++    0305,0309 = 0309,0320
++-+-+-- ---+++++    0305,0313 = 0313,0320
++-+-+-- ---+-+++    0301,0305 = 0301,0320 = 030103,0320 =
                     0317,0319 = 0319,0320
++-+--+- ---+-+++    0301,0306 = 030103,0306 = 0306,0319
++-+---+ ---+-+++    0301,0315 = 030103,0315 = 0315,0319 =
                     0316,0319
++-+---+ ---+--++    0303,0316 = 0315,0316
++-+--- -+-+-+++    0303,0309 = 0309,0319
++-+--- --++-+++    0301,0312 = 030103,0312 = 0310,0319 =
                     0312,0319
++-+--- ---+++++    0303,0313 = 0313,0319
++-+--- ---+-+++    0301,0303 = 0301,0319 = 030103,0303 =
                     030103,0319 = 0303,0319 = 0319,0319
++--+-- --++-+++    0301,0314 = 0304,0310
++--+-- --++-++-    030103,0314 = 0304,0314
++--+-- ---+-++-    030103,0304 = 0304,0304
++---+- ---+-+++    0301,0317 = 030103,0317
++------ -+-+-+++    0301,0309 = 030103,0309 = 0309,0309
++------ --++-+++    0301,0310 = 030103,0310
++------ ---+++++    0301,0313 = 030103,0313 = 0313,0313
++------ ---+-+++    0301,0301 = 0301,030103
```

```
-+-++---  --++-+++     0303,0314 = 0312,0314
-+-+-+--  --++-+++     0305,0310 = 0310,0320
-+-+----  --++-+++     0303,0310 = 0310,0312
--++-+-+  -+----++     0317,0318 = 0318,0320
--++-+-+  ------++     0307,0317 = 0307,0320
--++-+--  +-----++     0308,0317 = 0308,0320
--++-+--  -+----++     0311,0317 = 0311,0320
--++-+--  ------++     0302,0317 = 0302,0320
--++---+  -+----++     0303,0318 = 0311,0315 = 0315,0318
--++---+  -+----+-     0302,0318 = 0307,0311 = 0307,0318 =
                       0311,0318 = 0318,0318
--++---+  ------++     0302,0315 = 0303,0307 = 0307,0315
--++---+  ------+-     0302,0307 = 0307,0307
--++----  +-----+-     0302,0308 = 0308,0308
--++----  -+----+-     0302,0311 = 0311,0311
---+-++-  ------++     0305,0306 = 0306,0317 = 0306,0320
---+-+-+  ------++     0305,0315 = 0315,0317 = 0315,0320
---+-+--  --++--++     0305,0312 = 0312,0317 = 0312,0320
---+-+--  ------++     0303,0305 = 0303,0317 = 0303,0320 =
                       0305,0317 = 0305,0320 = 0317,0320 =
                       0320,0320
---+--+-  ------++     0303,0306 = 0306,0306
---+---+  ------++     0303,0315 = 0315,0315
---+----  --++--++     0303,0312 = 0312,0312

0301 = 030101 and 030102
```

LICENSES

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Notice to purchaser: Limited License.

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101.214-24/06 and 101.214-24u/06u

These products use ARMS™ technology and is sold under license from Zeneca Limited. ARMS is the subject of European Patent No. 0332435, US Patent No. 5595890 and corresponding world-wide patents. ARMS is a trademark of Zeneca Limited.

GUARANTEE

Olerup SSP AB guarantees that the primers in the DQB1*03 subtyping kit have the specificities given in the Specificity and Interpretation Tables of the product insert and in the GenoVision version of the HELMBERG-SCORE™ software.
When stored at –20°C, the dried primers are stable for 22 months from the date of manufacture.
When stored at –20°C, the PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* are stable for 24 months from the date of manufacture.
The kit is shipped at ambient temperature.

PROTOCOL

DNA EXTRACTION

Extracted, highly pure DNA is needed for SSP typings. We recommend isolation of DNA using GenoPrep B200 or GenoPrep B350 cartridges on the GenoM™-6 robotic workstation (GenoVision Europe *Tel:* +43 1 710 15 00 or GenoVision Inc. USA *Tel:* +1 610 430 88 41; http://www.genovision.com). Using GenoM™-6-extracted DNA ACD, EDTA and heparinised blood can be used as starting material. Because of its high purity, GenoM™-6-extracted DNA can be diluted when used in combination with *Olerup* SSP™ products. The recommended DNA concentration is 15 ng/ul.
Alternatively – BUT DO NOT USE HEPARINISED BLOOD WITH THESE METHODS - the DNA can be extracted using trimethylammoniumbromide salts (DTAB/CTAB) or by salting out. Dissolve the extracted DNA in dH_2O.

IMPORTANT:
Optimal DNA concentration using: GenoM™-6-extracted DNA, 15 ng/µl.
DNA extracted by other methods, 30 ng/µl.

Concentration exceeding 50 ng/µl will increase the risk for nonspecific amplifications and weak extra bands, especially for HLA Class I high resolution SSP typings.
weak extra bands, especially for HLA Class I high resolution SSP typings.

PCR AMPLIFICATION

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For one DQB1*03 subtyping, add at room temperature in a 0.5 ml tube:

19 x 2 µl = 38 µl DNA (30 ng/µl)
19 x 3 µl = 57 µl PCR Master Mix complete with *Taq* – mix well before taking your aliquot
19 x 5 µl = 95 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-H_2O mixture into each of the 16 wells of a DQB1*03 subtyping. ***Well No. 1 of the 16 well PCR plate is marked with '1'.*** Close the 16 well PCR plate with the provided lids.

101.214-24u/06u – <u>not</u> licensed for PCR

For one DQB1*03 subtyping, add at room temperature in a 0.5 ml tube:

19 x 2 µl = 38 µl DNA (30 ng/µl)
19 x 3 µl = 57 µl PCR Master Mix without *Taq* – mix well before taking your aliquot
1.5 µl µl *Taq* polymerase (5 units/µl)
19 x 5 µl – 1.5 µl = 93.5 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-*Taq*-H_2O mixture into each of the 16 wells of a DQB1*03 subtyping. ***Well No. 1 of the 16 well PCR plate is marked with '1'.*** Close the 16 well PCR plate with the provided lids.

Use a 96 well thermal cycler with a heated lid. The temperature gradient across the heating block should be < 1°C.

PCR cycling parameters:

1. 1 cycle	94°C	2 min	denaturation
2. 10 cycles	94°C	10 sec.	denaturation
	65°C	60 sec.	annealing and extension
3. 20 cycles	94°C	10 sec.	denaturation
	61°C	50 sec.	annealing
	72°C	30 sec.	extension

The same PCR cycling parameters are used for all the *Olerup* SSP kits.

AGAROSE GEL ELECTROPHORESIS

Prepare a 2% (w/v) agarose gel in 0.5 x TBE buffer. Dissolve the agarose by boiling in a microwave oven. Let the gel solution cool to 60°C. Stain the gel prior to casting with ethidium bromide (10 mg/ml), 5 µl per 100 ml gel solution. For maximal ease of handling use our ethidium bromide dropper bottles (Product No. 103.301-10), 1 drop of ethidium bromide solution per 50-75 ml of gel. **Note: Ethidium bromide is a powerful carcinogen.**

Load the PCR products, preferably using an 8-channel pipette. Load a DNA size marker (100 base pair ladder, Product No. 103.201-100) in one well per row.

Run the gel in 0.5 x TBE buffer, without re-circulation of the buffer, for 15-20 minutes at 8-10 V/cm.

DOCUMENTATION AND INTERPRETATION

Put the gel on a UV transilluminator and document by photography.

Record the presence and absence of specific PCR products. The relative lengths of the specific PCR products are helpful in the interpretation of the results.

Record the presence and relative lengths of the internal positive control bands. The differently sized control bands will help in the correct orientation of the typing as well as in kit identification.

Lanes without either control band or specific PCR products should be repeated.

Interpret the typings with the ***lot-specific Interpretation and Specificity Tables***.

INTERPRETATION SOFTWARE

The interpretation software (Product No. 110.101) can be helpful in the interpretation of the typings.

PCR MASTER MIXES

The PCR Master Mix complete with *Taq* contains:

Taq polymerase	0.4 unit per 10 μl SSP reaction
nucleotides	final concentration of each dNTP is 200 μM
PCR buffer	final concentrations: 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin
glycerol	final concentration of glycerol is 5%
cresol red	final concentration of cresol red is 100 μg/ml

The same PCR Master Mix complete with *Taq* is used for all the licensed *Olerup* SSP kits.

The PCR Master Mix without *Taq* contains:

nucleotides	final concentration of each dNTP is 200 μM
PCR buffer	final concentrations: 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin
glycerol	final concentration of glycerol is 5%
cresol red	final concentration of cresol red is 100 μg/ml

The same PCR Master Mix without *Taq* is used for all the unlicensed *Olerup* SSP kits.

The PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* can be shipped at ambient temperature.
When stored at –20°C, the PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* are stable for 24 months from the date of manufacture.
Vials with the PCR Master Mixes can be kept at +4°C for 4 weeks, but the PCR Master Mixes are then no longer stable for 24 months.

SPECIFICITY TABLE

DQB1*03 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for DQB1*03 SSP subtyping

Primer Mix	Approx. size of spec. PCR product[1]	Size of control band[2]	Amplified DQB1*03[3]	Other amplified DQB1 Alleles[4]
1	165 bp	**515 bp**	030101-030103, 0304, 0309, 0313, 0316, 0319	
2	220 bp	**515 bp**	030101-030103, 0304, 0309, 0310, 0313, 0314, 0316, 0319	
3[5]	135 bp	**515 bp**	030201-030204, 0307, 0308, 0311, 0318	020101-0202, 0204, 0205, 0629
4[6]	190, 220 bp	**515 bp**	030201-030204, 030302-030303, 030501-0308, 0311, 0312, 0315, 0318-0320	020101-0205, 0401, 0402
5	135 bp	430 bp	0304, 0314	
6[5,7]	95, 130 bp	430 bp	030501-030504, 0317, 0320	
7[5]	115 bp	430 bp	0306	
8[5,8]	110, 140, 220 bp	430 bp	0307, 0315, 0316, 0318	
9[5]	135 bp	430 bp	0308	060302
10[5,9]	135, 220, 260 bp	430 bp	0309, 0311, 0318	
11[5]	135 bp	430 bp	0310, 0312, 0314	060101-060103
12	260 bp	**515 bp**	030101-030103, 0304, 0309, 0310, 0312-0314, 0316, 0319	
13	165 bp	430 bp	0313	
14[5]	130 bp	**515 bp**	030101-030103, 0304, 0309, 0310, 0313, 0314, 0319	
15[5]	135 bp	**515 bp**	030101-0315, 0317-0320	
16[5]	130 bp	430 bp	030101-03010, 030302-030303, 0306, 0309, 0310, 0312, 0313, 0315,	

0317, 0319, 0320

[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*03 SSP subtypings.
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 bp or more. Size differences shorter than 20 bp are not given. For high resolution SSP kits the 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 band may sometimes be observed. Such bands can be disregarded and do not influence the interpretation of the SSP typings.
[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 tubes, or a band of 515 base pairs, for some tubes.
Tube 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*03 subtyping.
In addition, tubes number 2, 3, 4, 12, 14 and 15 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

PLEASE NOTE: All the SSP kits, except the B*37, B*41, B*42, B*46, B*47, B*48, B*49, B*50, B*53, B*67, B*78, B*81 and B*82 kits and the Cw*01, Cw*02, Cw*08, Cw*12,Cw*14, Cw*15, Cw*16, Cw*17 and Cw*18 kits, from *Olerup* SSP AB can be uniquely identified by the number of tubes and the kit-specific pattern of the two differently sized control bands.

In the presence of a specific amplification the intensity of the control band often decreases.
[3]For several DQB1 alleles only partial 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.
[4]Due to the sharing of sequence motifs, non-DQB1*03 alleles will be amplified by primer mixes 3, 4, 9 and 11.
[5]Specific PCR fragments shorter than 150 base pairs are less intense and not as sharp as longer specific bands.
[6]Primer mix 4: Specific PCR fragment of 190 bp in DQB1*030501-030504, 0319 and the DQB1*0401-0402 alleles. Specific PCR fragment of 220 bp in the DQB!*0306-0308, 0311, 01312, 0315, 0318, 0320 and the DQB1*020101-0205 alleles. Specific PCR fragments of 190 and 220 bp (both specific PCR fragments may not be obtained) in the DQB1*030201-030204 and DQB1*030302-030303 alleles.
[7]Primer mix 6: Specific PCR fragment of 95 bp in the DQB1*0620 allele. Specific PCR fragment of 130 bp in the DQB1*030501-030504 and 0317 alleles.
[8]Primer mix 8: Specific PCR fragment of 110 bp in the DQB1*0315 allele. Specific PCR fragment of 140 bp in the DQB1*0307 and 0316 alleles. Specific PCR fragment of 220 bp in the DQB1*0318 allele.
[9]Primer mix 10: Specific PCR fragment of 135 bp in the DQB1*0309 allele. Specific PCR fragment of 220 bp in the DQB1*0318 allele. Specific PCR fragment of 260 bp in the DQB1*0311 allele.

INTERPRETATION TABLE

DQB1*03 SSP subtyping

Amplification patterns of the DQB1*0301 to DQB1*0320 alleles

	Tube[5]							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Length of spec.	**165**	**220**	**135**	**190**	**135**	**95**	**115**	**110**
PCR product				**220**		**130**		**140**
								220
Length of int.	**515**	**515**	**515**	**515**	**430**	**430**	**430**	**430**
pos. control[1]								
5'-primer(s)[2]	**45**	**26**	**26**	**26**	**26**	**26**	**38**	**27**
	5'-g gA3'	**5'-T TA3'**	**5'-T CT3'**	**5'-T CT3'**	**5'-T TA3'**	**5'-g gg3'**	**5'-g CA3'**	**5'-TT C3'**
				135		**38**		**49**
				5'-T gA3'		**5'-AC A3'**		**5'-g gT3'**
3'-primer(s)[3]	**86**	**86**	**57**	**86**	**57**	**55**	**62**	**86**
	5'-g CT3'	**5'-g CT3'**	**5'-C gg3'**	**5'-g CT3'**	**5'-C gg3'**	**5'-g Cg3'**	**5'-CT A3'**	**5'-g CT3'**
				185				
				5'-Cg A3'				
Tube No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
DQB1 allele[4]								
***030101-030102**	**+**	**+**						
***030103**	**+**	**+**						
***030201-030204**			**+**	**+**				
***030302-030303**				**+**				
***0304**	**+**	**+**			**+**			
***030501-030504**				**+**		**+**		
***0306**				**+**			**+**	
***0307**			**+**	**+**				**+**
***0308**			**+**	**+**				
***0309**	**+**	**+**						
***0310**		**+**						
***0311**			**+**	**+**				
***0312**				**+**				
***0313**	**+**	**+**						
***0314**		**+**			**+**			
***0315**				**+**				**+**
***0316**	**+**	**+**						**+**
***0317**						**+**		
***0318**			**+**	**+**				**+**
Tube No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**

INTERPRETATION TABLE

DQB1*03 SSP subtyping

Amplification patterns of the DQB1*0301 to DQB1*0320 alleles

Tube[5]								
9	10	11	12	13	14	15	16	
135	135	135	260	165	130	135	130	Length of spec.
	220							PCR product
	260							
430	430	430	515	430	515	515	430	Length of int.
								pos. control[1]
38	14	13	13	26	26	55	57	5'-primer(s)[2]
5'-g CA3'	5'-gC C3'	5'-g gC3'	5'-g gC3'	5'-T TA3'	5'-T TA3'	5'-g CC3'	5'-T gA3'	
	27							
	5'-TT C3'							
	135							
	5'-T gA3'							
70	86	45	86	67	55	86	86	3'-primer(s)[3]
5'-CCC3'	5'-g CT3'	5'-CC C3'	5'-g CT3'	5'-g gT3'	5'-g Cg3'	5'-g CT3'	5'-g CT3'	
	167				55			
	5'-C AT3'				5'-g gg3'			
9	10	11	12	13	14	15	16	Tube No.
								DQB1 allele[4]
			+		+	+	+	*030101-030102
			+		+	+		*030103
						+		*030201-030204
						+	+	*030302-030303
			+		+	+		*0304
						+		*030501-030504
						+	+	*0306
						+		*0307
+						+		*0308
	+		+		+	+	+	*0309
		+	+		+	+	+	*0310
	+					+		*0311
		+	+			+	+	*0312
			+	+	+	+	+	*0313
		+	+		+	+		*0314
						+	+	*0315
			+					*0316
						+	+	*0317
	+					+		*0318
9	10	11	12	13	14	15	16	Tube No.

Length of spec.	**165**	**220**	**135**	**190**	**135**	**95**	**115**	**110**
PCR product				**220**		**130**		**140**
								220
Tube No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
***0319**	**+**	**+**		**+**				
***0320**				**+**		**+**		
***020101-0202, 0204, 0205**			**+**	**+**				
***0203, 0401, 0402**				**+**				
***060101-060103**								
***060302**								
***0629**			**+**					
DQB1 allele[4]								
Tube No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**

[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 tubes, or a band of 515 base pairs, for some tubes.
Tube 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*03 subtyping.
In addition, tubes number 2, 3, 4, 12, 14 and 15 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

PLEASE NOTE: All the SSP kits, except the B*37, B*41, B*42, B*46, B*47, B*48, B*49, B*50, B*53, B*67, B*78, B*81 and B*82 kits and the Cw*01, Cw*02, Cw*08, Cw*12,Cw*14, Cw*15, Cw*16, Cw*17 and Cw*18 kits, from *Olerup* SSP AB can be uniquely identified by the number of tubes and the kit-specific pattern of the two differently sized control bands.

135	135	135	260	165	130	135	130	Length of spec.
	220							PCR product
	260							
9	10	11	12	13	14	15	16	Tube No.
			+		+	+	+	*0319
						+	+	*0320
								*020101-0202, 0204, 0205
								*0203, 0401, 0402
		+						*060101-060103
+								*060302
								*0629
								DQB1 allele[4]
9	10	11	12	13	14	15	16	Tube No.

[2]The codon, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Codon numbering as in *Tissue Antigens* 1998, **51:II**, 467-507. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.
[3]The codon, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Codon numbering as in *Tissue Antigens* 1998, **51:II**, 467-507. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.
[4]The sequence of the DQB1*030301 allele has been shown to be identical to DQB1*030302.
[5]Primer mix 4: Specific PCR fragment of 190 bp in DQB1*030501-030504, 0319 and the DQB1*0401-0402 alleles. Specific PCR fragment of 220 bp in the DQB!*0306-0308, 0311, 01312, 0315, 0318, 0320 and the DQB1*020201-0205 alleles. Specific PCR fragments of 190 and 220 bp (both specific PCR fragments may not be obtained) in the DQB1*030201-030204 and DQB1*030302-030303 alleles.
Primer mix 6: Specific PCR fragment of 95 bp in the DQB1*0620 allele. Specific PCR fragment of 130 bp in the DQB1*030501-030504 and 0317 alleles.
Primer mix 8: Specific PCR fragment of 110 bp in the DQB1*0315 allele. Specific PCR fragment of 140 bp in the DQB1*0307 and 0316 alleles. Specific PCR fragment of 220 bp in the DQB1*0318 allele.
Primer mix 10: Specific PCR fragment of 135 bp in the DQB1*0309 allele. Specific PCR fragment of 220 bp in the DQB1*0318 allele. Specific PCR fragment of 260 bp in the DQB1*0311 allele.

CELL LINE VALIDATION SHEET

DQB1*03 SSP subtyping kit

					Tube															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Production No.	200622001	200622002	200622003	200736104	200736105	200736106	200622007	200622008	200622009	200622010	200622011	200622012	200622013	200736114	200622015	200622016
	cell line		DQB1																	
1	9001	SA	*0501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*0601	*0202	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-
3	9011	E4181324	*0601		-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
4	9275	GU373	*0201		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*0502		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*0302	*0601	-	-	+	+	-	-	-	-	-	-	+	-	-	-	+	-
7	9020	QBL	*0201		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
8	9007	DEM	*0302	*0502	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-
9	9026	YAR	*0302		-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-
10	9107	LKT3	*0401		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*0202		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0303		-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+
13	9067	BTB	*0402		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*0402		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*0303		-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+
16	9037	SWEIG007	*0301		+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+
17	9008	WILJON	*0602	*0603	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*0602	*0202	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*0301		+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+
20	9059	SLE005	*0604		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*0301		+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+
22	9056	KOSE	*0503	*0604	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*0503	*0601	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
24	9035	JBUSH	*0301		+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+
25	9049	IBW9	*0202		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*0201		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*0401	*0501	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*0202	*0301	+	+	+	+	-	-	-	-	-	-	-	+	-	+	+	+
29	9050	MOU	*0202		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*0402		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*0201		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*0301		+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+
33	9098	MT14B	*0302		-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-
34	9104	DHIF	*0301		+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+
35	9302	SSTO	*0305		-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
36	9024	KT17	*0302		-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-
37	9065	HHKB	*0603		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*0301		+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+
39	9315	CML	*0201	*0301	+	+	+	+	-	-	-	-	-	-	-	+	-	+	+	+
40	9134	WHONP199	*0202	*0303	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+
41	9055	H0301	*0609		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*0601		-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
43	9076	T7526	*0303		-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+
44	9057	TEM	*0503		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*0202		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*0301		+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+
48	9303	TER-ND	*0501			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP™ DQB1*03 SSP

Product number: **101.214-24/06 – licensed for PCR**
101.214-24u/06u – not licensed for PCR
Lot number: **Y07**
Expiry date: **2009-July-01**
Number of tests: **24 test – Product No. 101.214-24**
6 tests – Product No. 101.214-06
Number of tubes per test: **16**

Tube specifications:

Tube No.	Production No.	Tube No.	Production No.
1	2006-220-01	9	2006-220-09
2	2006-220-02	10	2006-220-10
3	2006-220-03	11	2006-220-11
4	2007-361-04	12	2006-220-12
5	2007-361-05	13	2006-220-13
6	2007-361-06	14	2007-361-14
7	2006-220-07	15	2006-220-15
8	2006-220-08	16	2006-220-16

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

No DNAs carrying the alleles to be amplified by primer solutions 7, 8, 9, 10 and 13 were available. The specificities of the primers in primer solutions 7 and 9 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 8 it was only possible to test the 3'-primer, the 5'-primers were not possible to test. In primer solution 10 one of the 3'-primers was not possible to test. In primer solution 13 it was only possible to test the 3'-primer, the 5'-primer was not possible to test.

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

Date of approval: 2007-September-12

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP™ DQB1*03
Product number: 101.214-24/06, 101.214-24u/06u
Lot number:

Intended use: DQB1*03 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 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
2007-September-12

Olle Olerup
Managing Director

WARRANTY

Olerup SSP AB warrants its products to the original purchaser against defects in materials and workmanship under normal use and application. *Olerup* SSP AB's sole obligation under this warranty shall be to replace, at no charge, any product that does not meet the performance standards stated on the product specification sheet.

This warranty applies only to products that have been handled and stored in accordance with *Olerup* SSP AB's recommendations, and does not apply to products that have been the subject of alternation, misuse, or abuse.

All claims under this warranty must be directed to *Olerup* SSP AB in writing and must be accompanied by a copy of the purchaser's invoice. This warranty is in lieu of all other warranties, expressed or implied, including the warranties of merchantability and fitness for a particular purpose. In no case shall *Olerup* SSP AB be liable for incidental or consequential damages.

This product may not be reformulated, repacked or resold in any form without the written consent of *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

Handle all samples as if capable of transmitting disease. All work should be performed wearing gloves and appropriate protection.

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