Olerup SSP™ DQA1

Product number:	**101.231-24/04 – licensed for PCR** **101.231-24u/04u – not licensed for PCR**
Lot number:	**Y46**
Expiry date:	**2009-October-01**
Number of tests:	**24 tests – Product No. 101.231-24** **4 tests – Product No. 101.231-04**
Number of tubes per test:	**32**
Storage - pre-aliquoted primers:	**dark at -20°C**
- PCR Master Mix:	**-20°C**

This Product Description is only valid for Lot No. Y46.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP™ DQA1 LOT

The DQA1 specificity and interpretation tables are unchanged compared the previous *Olerup* SSP™ DQA1 lot **(Lot No. X27)**, as no new DQA1 alleles have been described since the previous lot was made.

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

Tube	5'-primer	3'-primer	rationale
31	Exchanged	-	Longer specific PCR product.

Change in revision R01 compared to R00:

1. The DQA1*0509 allele is weakly amplified by primer mix 25.

PRODUCT DESCRIPTION

DQA1 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DQA1*0101 to DQA1*0602 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.
32 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.

STRIP LAYOUT

Each test consists of 32 PCR reactions in a 32 well cut PCR plate.

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**	**31**	**32**

The 32 well cut PCR plate is marked with 'DQA1 Y46'.
Well No. 1 is marked with '1'.
The PCR plates are heat-sealed with a PCR-compatible foil.
Please note: When removing each 32 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 DQA1 alleles will be amplified by the DQA1 typing kit. Thus, the interpretation of DQA1 typings is not influenced by the DQA2 gene.

UNIQUELY IDENTIFIED ALLELES

All the DQA1 alleles, i.e. **DQA1*0101 to 0107, DQA1*0201, DQA1*0301 to 0303, DQA1*0401 to DQA1*0404, DQA1*0501 to 0509 and DQA1*0601 to DQA1*0602**, recognized by the HLA Nomenclature Committee in October 2007[1] will give rise to unique amplification patterns by the primers in the DQA1 typing kit.

The DQA1 typing kit cannot distinguish the DQA1*010101 and 010102 alleles, the DQA1*010201 to 010203 alleles, the DQA1*010401 and 010402 alleles, the DQA1*050101 and 050102 alleles and the DQA1*060101 and 060102 alleles.

[1]***Nomenclature for factors of the HLA system, 1998***. *Tissue Antigens* 1999: **53**: 407-446.
DQA1 alleles listed on the IMGT/HLA web page 2007-October-05, release 2.19.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The DQA1 alleles give rise to 28 different amplification patterns, the DQa1*010201-010203 and 010204 alleles and the DQA1*040101 and 040102 alleles have different amplification patterns, which can be combined in 406 homozygous and heterozygous combinations. Twenty-seven of these genotypes do not give rise to unique amplification patterns.

```
++--++-- ----+--- -------+ --------    0104,0104 = 0104,0105
++------+ ----+++- +-+-+--+ +-------    0101,0509 = 0107,0505
                                        = 0107,0509
++------+ ----+--- -------+ +-------    0101,0107 = 0107,0107
-++---++ ----+--- -------+ --------    0102,0106 = 0106,0106
-------+ -+++---- -------+ --------    0302,0302 = 0302,0303
-------+ ----++++ +-+----+ --------    0501,0502 = 0502,0502
-------+ ----+++- +-+-+--- +-------    0505,0509 = 0509,0509
-------+ ----+++- +-+-+--- -------+    0505,0508 = 0508,0508
-------+ ----+++- -++----+ -----+--    0503,0506 = 0506,0506
-------+ ----+++- -++----+ ------+-    0503,0507 = 0507,0507
-------+ ----++-- ------++ -+------    0401,0402 = 0402,0402
-------+ ----++-- ------++ ---+----    0401,0404 = 0404,0404
-------+ ----+--- -----+++ ----+---    0601,0602 = 0602,0602
```

LICENSES

101.231-24/04 – licensed for PCR.
Notice to purchaser: Limited License.

The purchase price of this product includes limited, non-transferable rights under U.S. Patents 4,683,202, 4,683,195 and 4,965,188 and their foreign counterparts, owned by Roche Molecular Systems, Inc. and F. Hoffman-La Roche Ltd ("Roche"), to use only this amount of the product to practice the Polymerase Chain Reaction ("PCR") Process described in said patents solely for the HLA Typing applications of the purchaser solely for organ or tissue or bone marrow transplantation, and explicitly excludes analysis of forensic evidence or parentage determination. The rights to use this product to perform and to offer commercial service for HLA Typing for organ or tissue transplantation using PCR, including reporting the results of the purchaser's activities for a fee or other commercial consideration, is also hereby granted. Further information on purchasing licenses to practice PCR may be obtained by contacting in the United States, the Director of Licensing at Roche Molecular Systems, inc., 1145 Atlantic Avenue, Alameda, California 94501, and outside the United States, the PCR Licensing Manager, F. Hoffmann-La Roche Ltd, Grenzacherstr. 124, CH-4070 Basel, Switzerland.

101.231-24u/04u – not licensed for PCR.
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101.231-24/04 and 101.231-24u/04u

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 DQA1 typing 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/□l.
DNA extracted by other methods, 30 ng/□l.

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

PCR AMPLIFICATION

101.231-24/04 – licensed for PCR

For one DQA1 typing, add at room temperature in a 0.5 ml tube:

38 x 2 μl = 76 μl DNA (30 ng/μl)
38 x 3 μl = 114 μl PCR Master Mix complete with *Taq* – mix well before taking your aliquot
38 x 5 μl = 190 μl dH_2O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-H_2O mixture into each of the 32 wells of a DQA1 typing. ***Well No. 1 of the 32 well PCR plate is marked with '1'.*** Close the 32 well PCR plate with the provided lids.

101.231-24u/04u – <u>not</u> licensed for PCR

For one DQA1 typing, add at room temperature in a 0.5 ml tube:

38 x 2 μl = 76 μl DNA (30 ng/μl)
38 x 3 μl = 114 μl PCR Master Mix without *Taq* – mix well before taking your aliquot
3.0 μl *Taq* polymerase (5 units/μl)
38 x 5 μl – 3.0 μl = 187 μl dH_2O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-*Taq*-H_2O mixture into each of the 32 wells of a DQA1 typing. ***Well No. 1 of the 32 well PCR plate is marked with '1'.*** Close the 32 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

DQA1 SSP typing

Specificities and sizes of the PCR products of the 32 primer mixes used for DQA1 SSP typing

Primer Mix	Approx. size of spec. PCR product[1]	Size of control band[2]	Amplified DQA1 alleles[3]
1[4]	145 bp	**515 bp**	010101-010102, 010401-010402, 0105, 0107
2	170 bp	**515 bp**	010101-010102, 010201-010204, 010401-010402, 0105, 0106, 0107
3[4]	145 bp	430 bp	010201-010204, 0103, 0106
4	170 bp	430 bp	0103
5	220 bp	430 bp	010401-010402, 0105, 0106[?], 0107[?]
6[4]	100 bp	430 bp	010401-010402, 0106[?], 0107[?]
7[4]	95 bp	430 bp	0106
8[4]	65 bp	430 bp	010101-010203, 0103, 0106[?], 0107[?], 0201-0602
9	175 bp	430 bp	0201
10	185 bp	430 bp	030101, 0302, 0303
11[5]	215 bp	430 bp	0302
12[5]	225 bp	**515 bp**	0302, 0303
13	225 bp	**515 bp**	010101-030101, 040101, 0402-0602
14[4]	125 bp	430 bp	040101-040102, 0402, 0404, 050101-0509
15	165 bp	430 bp	050101-0509
16[4]	90 bp	430 bp	0502
17[5]	200 bp	430 bp	050101-050102, 0502[?], 0504[?], 0505, 0508, 0509
18[5]	200 bp	430 bp	0502[?], 0503, 0504[?], 0506, 0507
19	205 bp	430 bp	050101-0503, 0505-0509
20[4]	130 bp	430 bp	0504
21[4,5,6]	110 bp	430 bp	0502[?], 0504[?], 0505, 0508, 0509
22[4]	110 bp	**515 bp**	060101-060102, 0602
23[4,5]	85 bp	430 bp	040101-0404, 060101-0602
24[5]	240 bp	430 bp	010101-0107, 0201[weakly], 030101, 0302-050102, 0502[?], 0503, 0504[?], 0506, 0507, 060101-0602
25[7]	175, 275 bp	430 bp	0107, 0509[weakly]

26	150 bp	430 bp	0402
27[4,5]	90 bp	430 bp	0403N
28[4]	105 bp	430 bp	0404
29	160 bp	430 bp	0602
30	215 bp	430 bp	0506
31[4,5]	100 bp	430 bp	0507
32[4]	135 bp	430 bp	0508

[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 DQA1 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 15 bp or more. Size differences shorter than 15 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, e.g. the primers in tubes 11, 12, 17, 18, 21, 23 and 27.
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 DQA1 typing.
In addition, tubes number 2, 12, 13 and 22 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 DQA1 alleles only partial 1st, 2nd, 3rd and 4th exon nucleotide sequences are available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. We assume that unknown sequences are conserved within allelic groups.
[4]Short specific PCR fragments are less intense and not as sharp as longer specific bands.
[5]Primer mixes 11, 12, 17, 18, 21, 23, 24, 27 and 31 may give rise to nonspecific amplifications.
[6]Primer mix 21 may yield somewhat less specific PCR product than the other DQA1 primer mixes.
[7]Primer mix 25: Specific PCR product of 175 bp in the DQA1*0107 allele. Specific PCR product of 275 bp in the DQA1*0509 allele.
'?', nucleotide sequence information not available for the primer matching sequence.

INTERPRETATION TABLE

DQA1 SSP typing

Amplification patterns of the DQA1*0101 to *0602 alleles

	Tube[5]															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length of spec.	145	170	145	170	220	100	95	65	175	185	215	225	225	125	165	90
PCR product																
Length of int.	515	515	430	430	430	430	430	430	430	430	430	515	515	430	430	430
pos. control[1]																
5'-primer(s)[2]	34	25	34	25	-7	199	25	-7	7	7	-6	99	99	25	34	59
	5'-Ag g3'	5'-g TA3'	5'-Ag C3'	5'-g TT3'	5'-CC A3'	5'-gC A3'	5'-g TA3'	5'-CC g3'	5'-CAC3'	5'-CAT3'	5'-g AC3'	5'-CCC3'	5'-CCC3'	5'-g TA3'	5'-Ag C3'	5'-C Cg3'
								-7								
							5'-CC g3'									
3'-primer(s)[3]	69	69	69	69	1st in	218	44	2	52	55	1st in	160	160	53	75	75
	5'-TgC3'	5'-TgC3'	5'-TgC3'	5'-TgC3'	5'-TTT3'	5'-C TT3'	5'-AgC3'	5'-T gT3'	5'-T gT3'	5'-TCT3'	5'-TTT3'	5'-C AT3'	5'-C Ag3'	5'-TTg3'	5'-g AC3'	5'-g AC3'
													160			
													5'-C Ag3'			
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
DQA1 allele[4]																
***010101-010102**	+	+						+					+			
***010201-010203**		+	+					+					+			
***010204**		+	+										+			
***0103**			+	+				+					+			
***010401-010402**	+	+			+	+							+			
***0105**	+	+			+								+			
***0106**		+	+		?	?	+	?					+			
***0107**	+	+			?	?		?					+			
***0201**								+	+				+			
***030101**								+		+			+			
***0302**								+		+	+	+				
***0303**								+		+		+				
***040101**								+					+	+		
***040102**								+						+		
***0402**								+					+	+		
***0403N**								+					+			
***0404**								+					+	+		
***050101-050102**								+					+	+	+	
***0502**								+					+	+	+	+
***0503**								+					+	+	+	
***0504**								+					+	+	+	
***0505**								+					+	+	+	
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

INTERPRETATION TABLE

DQA1 SSP typing

Amplification patterns of the DQA1*0101 to *0602 alleles

Tube[5]																
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
200	200	205	130	110	120	85	240	175	150	90	105	160	215	100	135	Length of spec.
								275								PCR product
430	430	430	430	430	515	430	430	430	430	430	430	430	430	430	430	Length of int.
																pos. control[1]
107	107	21	21	-13	25	32	up[6]	up[6]	101	53	153	99	102	188	107	5'-primer(s)[2]
5'-C AT3'	5'-C AT3'	5'-T CC3'	5'-T CT3'	5'-gg A3'	5'-g TT3'	5'-gAC3'	5'-ACT3'	5'-ACT3'	5'-ACg3'	5'-gA T3'	5'-gT C3'	5'-CCC3'	5'-CA g3'	5'-CT A3'	5'-C AT3'	
								34								
								5'-Ag g3'								
160	160	75	52	1st in	52	47	-13	1	138	69	174	139	160	208	139	3'-primer(s)[3]
5'-AgC3'	5'-AgA3'	5'-g AC3'	5'-TCT3'	5'-TgC3'	5'-TCT3'	5'-ACA3'	5'-ggC3'	5'-TTT3'	5'-T gA3'	5'-TgT3'	5'-TC g3'	5'-gCg3'	5'-AgA3'	5'-gCA3'	5'-A gA3'	
								79								
								5'-gCA3'								
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.
																DQA1 allele[4]
							+									*010101
							+									*010201
							+									*010204
							+									*0103
							+									*010401
							+									*0105
							+									*0106
							+	+								*0107
							W									*0201
							+									*030101
							+									*0302
							+									*0303
						+	+									*040101
						+	+									*040102
						+	+		+							*0402
						+	+			+						*0403N
						+	+				+					*0404
+		+					+									*050101
?	?	+		?			?									*0502
	+	+					+									*0503
?	?		+	?			?									*0504
+		+		+												*0505
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.

Length of spec.	**145**	**170**	**145**	**170**	**220**	**100**	**95**	**65**	**175**	**185**	**215**	**225**	**225**	**125**	**165**	**90**
PCR product																
Tube No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
***0506**								**+**					**+**	**+**	**+**	
***0507**								**+**					**+**	**+**	**+**	
***0508**								**+**					**+**	**+**	**+**	
***0509**								**+**					**+**	**+**	**+**	
***060101-060102**								**+**					**+**			
***0602**								**+**					**+**			
DQA1 allele[4]																
Tube No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**

[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 DQA1 typing.
In addition, tubes number 2, 12, 13 and 22 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.

200	200	205	130	110	120	85	240	175	150	90	105	160	215	100	135	Length of spec.
								275								PCR product
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.
	+	+					+						+			*0506
	+	+					+							+		*0507
+		+		+											+	*0508
+		+		+				W								*0509
					+	+	+									*060101
					+	+	+					+				*0602
																DQA1 allele[4]
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.

[2]The codon, in the 1st, 2nd, 3rd or 4th 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 1st, 2nd, 3rd or 4th exon or the 1st intron, 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 DQA1*030102 allele has been shown to be identical to DQA1*0302.
DQA1*050103 has been renamed to DQA1*0505.
[5]Primer mix 25: Specific PCR product of 175 bp in the DQA1*0107 allele. Specific PCR product of 275 bp in the DQA1*0509 allele.
[6]Primer located upstream of the 1st exon.
'w', may be weakly amplified.
'?', nucleotide sequence information not available for the primer matching sequence.

CELL LINE VALIDATION SHEET

DQA1 SSP typing kit

					Tube															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.:	200629101	200629102	200629103	200629104	200629105	200629106	200629107	200629108	200629109	200629110	200629111	200629112	200629113	200629114	200629115	200629116
	cell line		DQA1*																	
1	9001	SA	*0101		+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-
2	9280	LK707	*0103	*0303	-	-	+	+	-	-	-	+	-	+	-	+	+	-	-	-
3	9011	E4181324	*0103		-	-	+	+	-	-	-	+	-	-	-	-	+	-	-	-
4	9275	GU373	*0501		-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-
5	9009	KAS011	*0102		-	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-
6	9353	SM	*0103	*0301	-	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-
7	9020	QBL	*0501		-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-
8	9007	DEM	*0303	*0102	-	+	+	-	-	-	-	+	-	+	-	+	+	-	-	-
9	9026	YAR	*0301		-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-
10	9107	LKT3	*0303		-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-
11	9051	PITOUT	*0201		-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
12	9052	DBB	*0201		-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
13	9067	BTB	*0401		-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-
14	9071	OLGA	*0401		-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-
15	9075	DKB	*0302		-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-
16	9037	SWEIG007	*0505		-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-
17	9008	WILJON	*0102		-	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-
18	9257	32367	*0102	*0303	-	+	+	-	-	-	-	+	-	+	-	+	+	-	-	-
19	9038	BM16	*0505		-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-
20	9059	SLE005	*0102		-	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-
21	9064	AMALA	*0503		-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-
22	9056	KOSE	*0102	*0104	+	+	+	-	+	+	-	+	-	-	-	-	+	-	-	-
23	9124	IHL	*0103	*0104	+	+	+	+	+	+	-	+	-	-	-	-	+	-	-	-
24	9035	JBUSH	*0505		-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-
25	9049	IBW9	*0201		-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
26	9285	WT49	*0501		-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-
27	9191	CH1007	*0303	*0105	+	+	-	-	+	-	-	+	-	+	-	+	+	-	-	-
28	9320	BEL5GB	*0201	*0303	-	-	-	-	-	-	-	+	+	+	-	+	+	-	-	-
29	9050	MOU	*0201		-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
30	9021	RSH	*0401		-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-
31	9019	DUCAF	*0501		-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-
32	9297	HAG	*0505		-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-
33	9098	MT14B	*0301		-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-
34	9104	DHIF	*0505		-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-
35	9302	SSTO	*0301		-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-
36	9024	KT17	*0301		-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-
37	9065	HHKB	*0103		-	-	+	+	-	-	-	+	-	-	-	-	+	-	-	-
38	9099	LZL	*0503		-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-
39	9315	CML	*0303	*0501	-	-	-	-	-	-	-	+	-	+	-	+	+	+	+	-
40	9134	WHONP199	*0201	*0302	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-
41	9055	H0301	*0102		-	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-
42	9066	TAB089	*0103		-	-	+	+	-	-	-	+	-	-	-	-	+	-	-	-
43	9076	T7526	*0302		-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-
44	9057	TEM	*0104		+	+	-	-	+	+	-	-	-		-	-	+	-	-	-
45	9239	SHJO	*0201	*0303	-	-	+	+	-	-	-	+	-	+	-	+	+	-	-	-
46	9013	SCHU	*0102		-	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-
47	9045	TUBO	*0505		-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-
48	9303	TER-ND	*0101		+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-

CELL LINE VALIDATION SHEET

DQA1 SSP typing kit

					Tube														
					17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
				Prod. No.:	200629117	200629118	200629119	200629120	200629121	200629122	200629123	200629124	200629125	200629126	200629127	200629128	200629129	200629130	200739831	200611332
	cell line		DQA1*																	
1	9001	SA	*0101		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
2	9280	LK707	*0103	*0303	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
3	9011	E4181324	*0103		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
4	9275	GU373	*0501		+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
5	9009	KAS011	*0102		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
6	9353	SM	*0103	*0301	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
7	9020	QBL	*0501		+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
8	9007	DEM	*0303	*0102	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
9	9026	YAR	*0301		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
10	9107	LKT3	*0303		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
11	9051	PITOUT	*0201		-	-	-	-	-	-	-	w	-	-	-	-	-	-	-	-
12	9052	DBB	*0201		-	-	-	-	-	-	-	w	-	-	-	-	-	-	-	-
13	9067	BTB	*0401		-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
14	9071	OLGA	*0401		-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
15	9075	DKB	*0302		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*0505		+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
17	9008	WILJON	*0102		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
18	9257	32367	*0102	*0303	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
19	9038	BM16	*0505		+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*0102		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
21	9064	AMALA	*0503		-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-
22	9056	KOSE	*0102	*0104	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
23	9124	IHL	*0103	*0104	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
24	9035	JBUSH	*0505		+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*0201		-	-	-	-	-	-	-	w	-	-	-	-	-	-	-	-
26	9285	WT49	*0501		+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
27	9191	CH1007	*0303	*0105	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*0201	*0303	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
29	9050	MOU	*0201		-	-	-	-	-	-	-	w	-	-	-	-	-	-	-	-
30	9021	RSH	*0401		-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
31	9019	DUCAF	*0501		+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
32	9297	HAG	*0505		+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*0301		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
34	9104	DHIF	*0505		+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*0301		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
36	9024	KT17	*0301		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
37	9065	HHKB	*0103		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
38	9099	LZL	*0503		-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-
39	9315	CML	*0303	*0501	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
40	9134	WHONP199	*0201	*0302	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
41	9055	H0301	*0102		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
42	9066	TAB089	*0103		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
43	9076	T7526	*0302		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
44	9057	TEM	*0104		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
45	9239	SHJO	*0201	*0303	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
46	9013	SCHU	*0102		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
47	9045	TUBO	*0505		+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*0101		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP™ DQA1 SSP

Product number: **101.231-24/04 – licensed for PCR**
101.231-24u/04u – not licensed for PCR
Lot number: **Y46**
Expiry date: **2009-October-01**
Number of tests: **24 tests – Product No. 101.231-24**
4 tests – Product No. 101.231-04
Number of tubes per test: **32**

Tube specifications:

Tube No.	Production No.	Tube No.	Production No.	Tube No.	Production No.
1	2006-291-01	13	2006-291-13	25	2006-291-25
2	2006-291-02	14	2006-291-14	26	2006-291-26
3	2006-291-03	15	2006-291-15	27	2006-291-27
4	2006-291-04	16	2006-291-16	28	2006-291-28
5	2006-291-05	17	2006-291-17	29	2006-291-29
6	2006-291-06	18	2006-291-18	30	2006-291-30
7	2006-291-07	19	2006-291-19	31	2007-398-31
8	2006-291-08	20	2006-291-20	32	2006-291-22
9	2006-291-09	21	2006-291-21		
10	2006-291-10	22	2006-291-22		
11	2006-291-11	23	2006-291-23		
12	2006-291-12	24	2006-291-24		

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 No. 7, 16, 20 and 25 to 32 were available. In primer solutions 7, 25, 26, 29, 31 and 3 the 5'-primers were tested by adding one additional 3-'primer, the 3'-primers were not possible to test. In prime solutions 16, 20, 27, 28 and 30 the 3'-primers were tested by adding one additional 5-'primer, the 5'-primers were not possible to test.

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

Date of approval: 2007-December-17

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP™ DQA1
Product number: 101.231-24/04, 101.231-24u/04u
Lot number: Y46

Intended use: HLA-DQA1 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
2007-December-17

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.

ADDRESSES:

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Web page: http://www.olerup.com

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For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.