

Olerup SSP[®] DQB1*02

Product number:	101.213-24 – including <i>Taq</i> polymerase
Lot number:	66E
Expiry date:	2010-June-01
Number of tests:	24
Number of Wells per test:	6
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. 66E.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP[®]* DQB1*02 Lot

The DQB1*02 specificity and interpretation tables have been updated with the DQB1 alleles described since the previous *Olerup SSP[®]* DQB1*02 lot (**Lot No. X56**) was made.

The DQB1*02 primer set is unchanged compared to the previous lot (**Lot No. X56**).

PRODUCT DESCRIPTION

DQB1*02 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DQB1*0201 to DQB1*0205 alleles.

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

PLATE LAYOUT

Each test consists of 6 PCR reactions in an 8 well PCR plate. Wells 7 to 8 are empty.

1	2	3	4	5	6	empty	empty
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The 8 well PCR plate is marked with 'DQB1*02'.

Well No. 1 is marked with the Lot No. '66E'.

The PCR plates are heat-sealed with a PCR-compatible foil.

Please note: When removing each 8 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Only DQB1 alleles will be amplified by the DQB1*02 subtyping kit. Thus, the interpretation of DQB1*02 subtypings is not influenced by the DQB2 and DQB3 genes.

UNIQUELY IDENTIFIED ALLELES

All the DQB1*02 alleles, i.e. **DQB1*0201 to DQB1*0205**, recognized by the HLA Nomenclature Committee in April 2008¹ will give rise to unique amplification patterns by the primers in the DQB1*02 subtyping kit.

The DQB1*02 kit cannot distinguish the DQB1*020101 and DQB1*020102 alleles.

¹HLA-DQB1 alleles listed on the IMGT/HLA web page 2008-April-08, release 2.21.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 5 DQB1*02 alleles can be combined in 15 homozygous and heterozygous combinations. All but two of these genotypes give rise to unique amplification patterns.

+-+-+- 0201,0204 = 0204,0204

+-+-+- 0201,0205 = 0205,0205

SPECIFICITY TABLE

DQB1*02 SSP subtyping

Specificities and sizes of the PCR products of the 6 primer mixes used for DQB1*02 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DQB1 ³ alleles
1	120	515 bp	020101-0202, 0204, 0205
2	85	430 bp	0203
3	145	515 bp	020101-020102, 0204, 0205, 030101-030204, 030302-0321, 0401-0403, 050101-0505, 060101-0634
4	140	430 bp	0202, 0203
5	145	430 bp	0204
6	180	430 bp	0205

¹Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of DQB1*02 SSP typings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective length of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an 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*02 subtyping.

In addition, well number 3 contains 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.

³For several DQB1 alleles only partial 3rd 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 the 3rd exon of DQB1 alleles are conserved within allelic groups.

INTERPRETATION TABLE						
DQB1*02 PCR-SSP subtyping						
Allele-specific amplification patterns of the 0201 to 0205 alleles						
	Well					
	1	2	3	4	5	6
Length of spec.	120	85	145	140	145	180
PCR product						
Length of int.	515	430	515	430	430	430
pos. control¹						
5'-primer²	30	57	135	102	136	30
	^{5'} -A Ag ^{3'}	^{5'} -T gA ^{3'}	^{5'} -T gA ^{3'}	^{5'} -TC T ^{3'}	^{5'} -AC g ^{3'}	^{5'} -A Ag ^{3'}
3'-primer³	57	71	170	135	170	77
	^{5'} -C gg ^{3'}	^{5'} -Cg T ^{3'}	^{5'} -gAC ^{3'}	^{5'} -g gC ^{3'}	^{5'} -gAC ^{3'}	^{5'} -CCg ^{3'}
Well No.	1	2	3	4	5	6
DQB1 allele⁴						
*020101-020102	1		3			
*0202	1			4		
*0203		2		4		
*0204	1		3		5	
*0205	1		3			6
*030101-030204, 030302-0321, 0401-0403, 050101-0505, 060101-0634			3			
DQB1 allele⁴						
Well No.	1	2	3	4	5	6

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*02 subtyping.

In addition, well number 3 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

²The codon, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.

³The 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 on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.

⁴For several DQB1 alleles only partial 3rd 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 the 3rd exon of DQB1 alleles are conserved within allelic groups.

CELL LINE VALIDATION SHEET										
DQB1*02 SSP subtyping kit										
				Production No.	Well					
					1	2	3	4	5	6
					200620401	200620402	200620403	200620404	200620405	200731706
	IHWC 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*02 SSP

Product number: 101.213-24 – including *Taq* polymerase
Lot number: 66E
Expiry date: 2010-June-01
Number of tests: 24
Number of wells per test: 6

Well specifications:

Well No.	Production No.
1	2006-204-01
2	2006-204-02
3	2006-204-03
4	2006-204-04
5	2006-204-05
6	2007-317-06

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

No DNAs carrying the alleles to be amplified by primer solutions No. 2, 5 and 6 were available. The specificities of the primers in these primer solutions were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer.

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

Date of approval: 2009-May-25

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP[®] DQB1*02
Product number: 101.213-24
Lot number: 66E

Intended use: DQB1*02 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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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
2009-May-25

Olle Olerup
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