101.215-12 – including *Taq* pol., IFU-01
101.215-12u – without *Taq* pol., IFU-02

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"Instructions for Use" (IFU)

Lot No.: 62R

Lot-specific Information

Olerup SSP® DQB1*04

Product number:	**101.215-12 – including *Taq* polymerase** **101.215-12u – without *Taq* polymerase**
Lot number:	**62R**
Expiry date:	**2015-May-01**
Number of tests:	**12**
Number of wells per test:	**8**
Storage - pre-aliquoted primers:	**dark at -20°C**
- PCR Master Mix:	**-20°C**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 62R.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® DQB1*04 LOT

The DQB1*04 specificity and interpretation tables have been updated with the DQB1 alleles described since the previous *Olerup* SSP® DQB1*04 lot **(Lot No. 87M)** was made.

The Lot-specific information for DQB1*04 including and without *Taq* polymerase is now described in one common Product Insert.

The DQB1*04 primer set is unchanged compared to the previous *Olerup* SSP® DQB1*04 lot **(Lot No. 87M)**.

101.215-12 – including *Taq* pol., IFU-01
101.215-12u – without *Taq* pol., IFU-02

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Lot No.: 62R **Lot-specific Information**

PRODUCT DESCRIPTION

DQB1*04 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DQB1*04:01 to DQB1*04:08 alleles.

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

PLATE LAYOUT

Each test consists of 8 PCR reactions in an 8 well PCR plate.

1	2	3	4	5	6	7	8

The 8 well PCR plate is marked with 'DQ4' in silver/gray ink.
Well No. 1 is marked with the Lot No. '62R'.
A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.
The PCR plates are covered with a PCR-compatible foil.
Please note: When removing each 8 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Only the DQB1*04 alleles will be amplified by the DQB1*04 subtyping kit, except DQB1*03 alleles will be amplified by primer mixes 4, 5 and 8. Thus, the interpretation of DQB1*04 subtypings is not influenced by other groups of the DQB1 alleles or the DQB2 and DQB3 genes.

UNIQUELY IDENTIFIED ALLELES

All the DQB1*04 alleles, i.e. **DQB1*04:01 to DQB1*04:08**, recognized by the HLA Nomenclature Committee in July 2012[1] will give rise to unique amplification patterns by the primers in the DQB1*04 subtyping kit.

The DQB1*04 subtyping kit cannot distinguish the silent mutations in the DQB1*04:01:01 and DQB1*04:01:02 alleles or the DQB1*04:02:01 and 04:02:02 alleles.

[1]HLA-DQB1 alleles listed on the IMGT/HLA web page 2012-July-12, release 3.9.0, www.ebi.ac.uk/imgt/hla.

Lot No.: 62R **Lot-specific Information**

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 11 alleles generate 9 amplification patterns that can be combined in 45 homozygous and heterozygous combinations. 19 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.

+−+−+−−−	*04:02:01, *04:04 = *04:04, *04:04
+−++−−−−	*04:02:01, *04:03:01 = *04:02:01, *04:03:02 = *04:03:01, *04:03:01 = *04:03:01, *04:03:02
++−−−−−+	*04:01:01, *04:08 = *04:08, *04:08
++−−−−+−	*04:01:01, *04:07 = *04:07, *04:07
++−−−+−−	*04:01:01, *04:06 = *04:06, *04:06
++−−+−−−	*04:01:01, *04:05 = *04:05, *04:05
+−+++−−−	*04:03:01, *04:04 = *04:03:02, *04:04
+++−+−−−	*04:01:01, *04:04 = *04:02:01, *04:05 = *04:04, *04:05
+++−+−−−	*04:01:01, *04:04 = *04:02:01, *04:05 = *04:04, *04:05
++−−+−−−	*04:01:01, *04:05 = *04:05, *04:05
++−−−+−−	*04:01:01, *04:06 = *04:06, *04:06
++−−−−+−	*04:01:01, *04:07 = *04:07, *04:07
++−−−−−+	*04:01:01, *04:08 = *04:08, *04:08
+−+++−−−	*04:03:01, *04:04 = *04:03:02, *04:04
+−++−−−−	*04:02:01, *04:03:01 = *04:02:01, *04:03:02 = *04:03:01, *04:03:01 = *04:03:01, *04:03:02
+−+−+−−−	*04:02:01, *04:04 = *04:04, *04:04

*04:01:01 = *04:01:01 and 04:01:02
*04:02:01 = *04:02:01 and 04:02:02

101.215-12 – including *Taq* pol., IFU-01
101.215-12u – without *Taq* pol., IFU-02

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Lot No.: 62R

Lot-specific Information

SPECIFICITY TABLE

DQB1*04 SSP subtyping

Specificities and sizes of the PCR products of the 8 primer mixes used for DQB1*04 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified DQB1*04 alleles[3]	Amplified non-DQB1*04 alleles[4]
1[6,7]	210 bp, 245 bp	**515 bp**	*04:01:01-04:08	
2[6]	205 bp	**515 bp**	*04:01:01-04:01:02, 04:05-04:08	
3	205 bp	430 bp	*04:02:01-04:03:01, 04:04	
4	195 bp	430 bp	*04:03:01-04:03:02	*03:06, 03:25
5	245 bp	430 bp	*04:04-04:05	*03:06, 03:25
6[5]	110 bp	430 bp	*04:06	
7	160 bp	430 bp	*04:07	
8[5]	95 bp	430 bp	*04:08	*03:06[?]-03:08[?], 03:11[?]-03:18[?], 03:19, 03:20[?], 03:23[?], 03:26[?], 03:37[?], 03:40[?], 03:44[?]

[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*04 SSP typings.
When the primers in a primer mix can give rise to HLA-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits the respective lengths of the HLA-specific PCR product(s) are given for the alleles amplified by these primer mixes.
Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.
PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.
PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.
Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherit feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.
[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 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*04 subtyping.

Lot No.: 62R **Lot-specific Information**

In addition, well number 2 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.
[3]For several DQB1 alleles 1st and/or 3rd exon(s) and beyond, as well as intron nucleotide sequences, are not 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 in these regions are conserved within allelic groups.
[4]Due to the sharing of sequence motifs between DQB1 alleles non-DQB1*04 alleles will be amplified by primer mixes 4, 5 and 8.
[5]HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.
[6]Primer mixes 1 and 2 may give rise to a lower yield of HLA-specific PCR product than the other DQB1*04 primer mixes.
[7]Primer mix 1: Specific PCR fragment of 210 bp in the DQB1*04:04 and 04:05 alleles. Specific PCR fragment of 245 bp in the DQB1*04:02:02 and 04:03:02 alleles. Specific PCR fragments of 210 and 245 bp in the DQB1*04:01:01-04:02:01, 04:03:01 and 04:06-04:08 alleles.
'?', nucleotide sequence information not available for the primer matching sequence.

101.215-12 – including *Taq* pol., IFU-01
101.215-12u – without *Taq* pol., IFU-02

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Lot No.: 62R **Lot-specific Information**

INTERPRETATION TABLE

DQB1*04 SSP subtyping

Amplification patterns of the DQB1*04 alleles

	Well[4]							
	1	2	3	4	5	6	7	8
Length of spec.	210	205	205	195	245	110	160	95
PCR product(s)	245							
Length of int.	515	515	430	430	430	430	430	430
pos. control[1]								
5'-primer[2]	9(122)	23(164)	23(164)	26(173)	9(122)	54(259)	144(529)	167(596)
	5' -gTT 3'	5' -gCT 3'	5' -gCg 3'	5' -TCT 3'	5' -gTA 3'	5' -ggT 3'	5' -CCg 3'	5' -gCA 3'
	21(159)		23(164)					
	5' -ACC 3'		5' -gCg 3'					
3'-primer[3]	77(327)	77(327)	77(327)	77(327)	77(327)	77(327)	185(650)	185(650)
	5' -ACg 3'	5' -ACg 3'	5' -ACg 3'	5' -ACg 3'	5' -ACg 3'	5' -ACg 3'	5' -CgA 3'	5' -CgA 3'
Well No.	1	2	3	4	5	6	7	8
DQB1 allele								
*04:01:01-04:01:02	1	2						
*04:02:01-04:02:02	1		3					
*04:03:01	1		3	4				
*04:03:02	1			4				
*04:04	1		3		5			
*04:05	1	2			5			
*04:06	1	2				6		
*04:07	1	2					7	
*04:08	1	2						8
*03:06				4	5			?
*03:07-03:08, 03:11-03:18, 03:20, 03:23, 03:26, 03:37, 03:40, 03:44								?
*03:19								8
*03:25				4	5			
DQB1 allele								
Well No.	1	2	3	4	5	6	7	8

101.215-12 – including *Taq* pol., IFU-01
101.215-12u – without *Taq* pol., IFU-02

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Lot No.: 62R **Lot-specific Information**

[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 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*04subtyping.
In addition, well number 2 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.
[2]The codon, and in parenthesis the nucleotide, in the 2nd or 3rd exon, 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.
[3]The codon, and in parenthesis the nucleotide, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. 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.
[4]Primer mix 1: Specific PCR fragment of 210 bp in the DQB1*04:04 and 04:05 alleles. Specific PCR fragment of 245 bp in the DQB1*04:02:02 and 04:03:02 alleles. Specific PCR fragments of 210 and 245 bp in the DQB1*04:01:01-04:02:01, 04:03:01 and 04:06-04:08 alleles.
'?', nucleotide sequence information not available for the primer matching sequence.

101.215-12 – including *Taq* pol., IFU-01
101.215-12u – without *Taq* pol., IFU-02

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Lot No.: 62R **Lot-specific Information**

CELL LINE VALIDATION SHEET

DQB1*04 SSP subtyping kit

					Well							
					1	2	3	4	5	6	7	8
				Production No.	201077501	201077502	201192603	200846904	201077505	201192606	201192607	201192608
	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		-	-	-	-	-	-	-	-

101.215-12 – including *Taq* pol., IFU-01
101.215-12u – without *Taq* pol., IFU-02

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Lot No.: 62R **Lot-specific Information**

CERTIFICATE OF ANALYSIS

Olerup SSP® DQB1*04 SSP

Product number:	**101.215-12 – including *Taq* polymerase** **101.215-12u – without *Taq* polymerase**
Lot number:	**62R**
Expiry date:	**2015-May-01**
Number of tests:	**12**
Number of wells per test:	**8**

Well specifications:

Well No.	Production No.
1	2010-775-01
2	2010-775-02
3	2011-926-03
4	2008-469-04
5	2010-775-05
6	2011-926-06
7	2011-926-07
8	2011-926-08

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

No DNAs carrying the alleles to be amplified by primer solutions 4 to 7 were available. The specificities of the primers in primer solutions 4 and 5 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 6 and 7 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solution 3 one 5'-primer was not possible to test.

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

Date of approval: 2012-December-07

Approved by:

Production Quality Control

CE

Lot No.: 62R **Lot-specific Information**

Declaration of Conformity

Product name: *Olerup* SSP® DQB1*04
Product number: 101.215-12/12u
Lot number: 62R

Intended use: DQB1*04 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
2012-December-07

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

101.215-12 – including *Taq* pol., IFU-01
101.215-12u – without *Taq* pol., IFU-02

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Lot No.: 62R **Lot-specific Information**

Lot No.: 62R **Lot-specific Information**

ADDRESSES:

Manufacturer:
***Olerup* SSP AB**, Franzengatan 5, SE-112 51 Stockholm, Sweden.
Tel: +46-8-717 88 27
Fax: +46-8-717 88 18
E-mail: info-ssp@olerup-ssp.com
Web page: http://www.olerup.com

Distributed by:
Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.
Tel: +43-1-710 15 00
Fax: +43-1-710 15 00 10
E-mail: support-at@olerup.com
Web page: http://www.olerup.com

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382
Tel: 1-877-OLERUP1
Fax: 610-344-7989
E-mail: info.us@olerup.com
Web page: http://www.olerup.com

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