

101.901-24 – including *Taq* polymerase, IFU-01

101.901-24u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for

“Instructions for Use” (IFU)

Lot No.: **47X**

Lot-specific information

Olerup SSP® DQB1*06:02,DQA1*01:02 - SSP

Product number:	101.901-24 – including <i>Taq</i> polymerase 101.901-24u – without <i>Taq</i> polymerase
Lot number:	47X
Expiry date:	2017-September-01
Number of tests:	24
Number of wells per test:	7+1
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. 47X.

Complete product documentation consists of generic Instructions for Use (IFU), lot specific Product Insert, Worksheet and Certificate.

**CHANGES COMPARED TO THE PREVIOUS OLERUP SSP®
DQB1*06:02,DQA1*01:02 LOT (33S)**

The DQB1*06:02,DQA1*01:02 kit has been redesigned and improved with regards to allelic detection and discrimination and facilitated interpretation. The kit resolution focuses on common and well documented (CWD) alleles¹.

The new lot contains 8 primer mixes for the DQA1, DQB1 and DRB1 alleles and includes a negative control.

The format of the Product Insert and Worksheet have been changed.

The DQB1*06:02,DQA1*01:02 primer set, specificity and interpretation tables have been updated for the DQA1, DQB1 and DRB1 alleles described since the previous Olerup SSP® DQB1*06:02,DQA1*01:02 lot (**Lot No. 33S**) was made. The kit design is based on IMGT/HLA database 3.19.0.

¹S. J. Mack¹, P. Cano², J. A. Hollenbach¹ et al.
Common and well-documented HLA alleles: 2012 update to
the CWD catalogue. Tissue Antigens, 2013, 81, 194–203

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As of lot series V, the Specificity Table is included in the lot-specific Product Insert, and the Interpretation Table is included in the Worksheet.

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

Well	5'-primer	3'-primer	rationale
1	New	New	New primer pair for separation of the DQA1*01:01 from DQA1*01:02 alleles
2	Changed well position	Changed well position	Primer pair for the DQA1*01:01 and DQA*01:02 alleles, previously well 1.
3	Changed well position	Changed well position	Primer pair for the DQA1*01:02 and DQA*01:03 alleles, previously well 2.
4	-	-	Primer pair for separation of the DQA1*01:02 from DQA1*01:03 alleles
5	Changed well position	Changed well position	Primer pair for the DQB1*06:02 alleles, previously well 8.
6	New	New	Primer pair for improved allelic resolution of the DQA1*06:02 alleles.
7	New	New	New primer pair for the DRB1*15 group of alleles.
8	-	-	Updated negative control.

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Well **8** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup* SSP® HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

HLA-specific PCR product sizes range from 75 to 200 base pairs.

The PCR product generated by the positive control primer pair is 430 base pairs.

Length of PCR product	105	200	105	80	75	80	85
5'-primer¹	164	340	440	45	45	43	36
	5'-CAC ^{3'}	5'-Agg ^{3'}	5'-TTA ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-TAC ^{3'}
							36
							5'-TAT ^{3'}
3'-primer²	231	2nd I	507	59	58	57	47
	5'-TgC ^{3'}	5'-AAA ^{3'}	5'-TTg ^{3'}	5'-CTC ^{3'}	5'-ggC ^{3'}	5'-CTC ^{3'}	5'-ACA ^{3'}
							48
							5'-gCA ^{3'}
							48
							5'-gCC ^{3'}
							52
							5'-TgT ^{3'}
A*	+	+	+				
B*	+	+	+				
C*	+	+	+				
DRB1				+	+		
DRB3				+	+		
DRB5				+			
DQB1					+		
DPB1						+	
DQA1							+

¹The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and 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.

²The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and 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.

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PRODUCT DESCRIPTION

DQB1*06:02,DQA1*01:02 - SSP

CONTENT

The primer set contains 5'- and 3'-primers for identifying the following on CWD level^{1,2}:

DQB1*06:02 DQA1*01:02 DRB1*15:xx

DQB1*06:02 DQA1*01:02

DQB1*06:02 DRB1*15:xx

DQB1*06:02

DQA1*01:02

DRB1*15:xx

¹Mahlis J et al.

The autoimmune basis of narcolepsy.

Current Opinion in Neurobiology 2013, 23:767–773

²Mignot E et al.

HLA DQB1*0602 is associated with cataplexy in 509 narcoleptic patients.

Sleep 1997, 20:1012-1020.

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 a 8 well PCR plate

1	2	3	4	5	6	7	8
DQA1	DQA1	DQA1	DQA1	DQB1	DQB1	DRB1	NC

The 8 well PCR plate is marked with '47X' in silver/gray ink.

Well No. 1 is marked with the Lot No. '47X'.

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.

Wells 1 to 4 – DQA1 high resolution primers.

Wells 5 to 6 – DQB1 high resolution primers.

Well 7 – DRB1 low resolution primers.

Well 8 – Negative Control (NC).

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.

UNIQUELY IDENTIFIED ALLELES

DQA1, DQB1 and DRB1 alleles recognized by the HLA Nomenclature Committee in January 2015^{1,2} have been considered in the specificity and interpretation tables of the DQB1*06:02,DQA1*01:02 kit.

For further details see Content section.

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The DQB1*06:02,DQA1*01:02 kit cannot distinguish the DQB1*06:02:01-06:02:04, 06:02-06:06:02:23 alleles and the DQA1*01:02:01:01-01:02:04 alleles.

¹DQA1, DQB1 and DRB1 alleles listed on the IMGT/HLA web page 2015-Janaury-19, release 3.19.0, www.ebi.ac.uk/imgt/hla.

²Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page <http://hla.alleles.org/alleles/deleted.html>.

EXPECTED RESULTS

Table 1 describes expected results for the (groups of) alleles that the kit is able to detect and separate.

Table 1: Expected results for targeted DQA1, DQB1 and DRB1 alleles.

DQA1 alleles	DQB1 alleles	DRB1 alleles	Positive DQA1 wells	Positive DQB1 wells ¹	Positive DRB1 wells
*01:02	*06:02	*15:xx	2, 3	5	7
	*06:02	*15:xx		5	7
*01:02	*06:02		2, 3	5	
*01:02		*15:xx	2, 3		7
	*06:02			5	
*01:02			2, 3		
		*15:xx			7

¹The DQB1*06:02:05 allele is also amplified in well 6.

In the negative control well no PCR product should be seen. The presence of PCR product(s) indicates contamination. Primer oligomer artifacts, approximately 40 to 50 bp in size, may be seen. This does not represent contamination.

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SPECIFICITY TABLE

DQB1*06:02,DQA1*01:02 - SSP

Specificities and sizes of the PCR products of the 7+1 primer mixes used for DQB1*06:02,DQA1*01:02 SSP typing

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA class I alleles ³
1 ⁴	145 bp	515 bp	DQA1*01:01:01-01:01:02, DQA1*01:04:01:01-01:05:02, DQA1*01:07, DQA1*01:12
2	170 bp	515 bp	DQA1*01:01:01-01:02:04, DQA1*01:04:01:01-01:09, DQA1*01:11-01:12
3 ⁴	145 bp	430 bp	DQA1*01:02:01:01-01:03:01:02, DQA1*01:06, DQA1*01:08-01:11
4	170 bp	430 bp	DQA1*01:03:01:01-01:03:01:02, DQA1*01:10
5	185 bp	430 bp	DQB1*04:10, DQB1*06:02:01-06:02:23, DQB1*06:14:01-06:16, DQB1*06:19:01-06:20, DQB1*06:23-06:24, DQB1*06:33, DQB1*06:37, DQB1*06:46-06:50, DQB1*06:51:02, DQB1*06:68, DQB1*06:70-06:84, DQB1*06:95, DQB1*06:97, DQB1*06:107, DQB1*06:109, DQB1*06:111-06:117, DQB1*06:122, DQB1*06:124-06:127, DQB1*06:136-06:138, DQB1*06:146-06:147, DQB1*06:150-06:152, DQB1*06:156, DQB1*06:159, DQB1*06:161-06:163, DQB1*06:166
6	155 bp	430 bp	DQB1*03:30, DQB1*03:72, DQB1*03:100, DQB1*03:132, DQB1*04:01:01-04:02:04, DQB1*04:02:06, DQB1*04:02:08-04:03:02, DQB1*04:06-04:08, DQB1*04:10, DQB1*04:12-04:14, DQB1*04:16-04:27, DQB1*05:38, DQB1*06:02:05, DQB1*06:19:01, DQB1*06:139
7	215 bp	430 bp	DRB1*15:01:01:01-15:114, DRB1*15:116-15:117
8 ⁵	-	-	Negative Control

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 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 alleles listed are specified according to amplicon length.

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.

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²The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 430 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

³For several DQA1, DQB1 and DRB1 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. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

⁴Primer mixes 1 and 3 may give rise to a lower yield of HLA-specific PCR product than the other DQB1*06:02,DQA1*01:02 primer mixes.

⁵Primer mix 8 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by the control primer pairs matching the human growth hormone gene. HLA-specific PCR product sizes range from 75 to 200 base pairs and the PCR product generated by the HGH positive control primer pair is 430 base pairs.

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DQA1 PRIMER SPECIFICATION

Well No.	1	2	3	4
Length of spec. PCR product	145	170	145	170
Length of int. pos. control ¹	515	515	430	430
5'-primer(s) ²	33(169) 5' -Agg 3'	25(143) 5' -gTA 3'	33(169) 5' -AgC 3'	25(143) 5' -gTT 3'
3'-primer(s) ³	68(274) 5' -TgC 3'	68(274) 5' -TgC 3'	68(274) 5' -TgC 3'	68(274) 5' -TgC 3'
Well No.	1	2	3	4

DQB1 PRIMER SPECIFICATION

Well No.	5	6
Length of spec. PCR product	185	155
Length of int. pos. control ¹	430	430
5'-primer(s) ²	9(122) 5' -gTT 3'	9(122) 5' -gTT 3'
3'-primer(s) ³	57(266) 5' -CAT 3'	47(237) 5' -CgA 3'
	58(270) 5' -TCC 3'	
Well No.	5	6

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DRB1 PRIMER SPECIFICATION

Well No.	7
Length of spec.	215
PCR product	
Length of int. pos. control ¹	430
5'-primer(s) ²	13(126)
	5' -Agg 3'
	13(126)
	5' -AAg 3'
	13(126)
	5' -AgA 3'
3'-primer(s) ³	66(286)
	5' -gAT 3'
	69(295)
	5' -CTg 3'
	69(295)
	5' -..Tg 3'
	70(298)
	5' -CgC 3'
	71(299)
	5' -gCT 3'
	73(305)
	5' -ggC 3'
	77(317)
	5' -AgT 3'
Well No.	7

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²The nucleotide position matching the specificity-determining 3'-end of the primer is given. 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 nucleotide position matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. 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.

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CELL LINE VALIDATION SHEET							
DQA1*01:02							
				Well			
				1	2	3	4
				Prod. No.	201443101B	201443101	201443102
					201443104		
IHWC cell line				DQA1			
1	9001	SA	*01:01		+	+	-
2	9280	LK707	*01:03	*03:03	-	-	+
3	9011	E4181324	*01:03		-	-	+
4	9275	GU373	*05:01		-	-	-
5	9009	KAS011	*01:02		-	+	+
6	9353	SM	*01:03	*03:01	-	-	+
7	9020	QBL	*05:01		-	-	-
8	9025	DEU	*03		-	-	-
9	9026	YAR	*03:01		-	-	-
10	9107	LKT3	*03:03		-	-	-
11	9051	PITOUT	*02:01		-	-	-
12	9052	DBB	*02:01		-	-	-
13	9004	JESTHOM	*01:01		+	+	-
14	9071	OLGA	*04:01		-	-	-
15	9075	DKB	*03:02		-	-	-
16	9037	SWEIG007	*05:05		-	-	-
17	9282	CTM3953540	*01:03	*05:01	-	-	+
18	9257	32367	*01:02	*03:03	-	+	+
19	9038	BM16	*05:05		-	-	-
20	9059	SLE005	*01:02		-	+	+
21	9064	AMALA	*05:03		-	-	-
22	9056	KOSE	*01:02	*01:04	+	+	+
23	9124	IHL	*01:03	*01:04	+	+	+
24	9035	JBUSH	*05:05		-	-	-
25	9049	IBW9	*02:01		-	-	-
26	9285	WT49	*05:01		-	-	-
27	9191	CH1007	*03:03	*01:05	-	+	-
28	9320	BEL5GB	*02:01	*03:03	-	-	-
29	9050	MOU	*02:01		-	-	-
30	9021	RSH	*04:01		-	-	-
31	9019	DUCAF	*05:01		-	-	-
32	9297	HAG	*05:05		-	-	-
33	9098	MT14B	*03:01		-	-	-
34	9104	DHIF	*05:05		-	-	-
35	9302	SSTO	*03:01		-	-	-
36	9024	KT17	*03:01		-	-	-
37	9065	HHKB	*01:03		-	-	+
38	9099	LZL	*05:03		-	-	-
39	9315	CML	*03:03	*05:01	-	-	-
40	9134	WHONP199	*02:01	*03:02	-	-	-
41	9055	H0301	*01:02		-	+	+
42	9066	TAB089	*01:03		-	-	+
43	9076	T7526	*03:02		-	-	-
44	9057	TEM	*01:04		+	+	-
45	9239	SHJO	*02:01	*03:03	-	-	-
46	9013	SCHU	*01:02		-	+	+
47	9045	TUBO	*05:05		-	-	-
48	9303	TER-ND	*01:01		+	+	-

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CELL LINE VALIDATION SHEET					
DQB1*06:02					
				Well	
				5	6
			Prod. No.	201443105	201443106
	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 SSTS	*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		-	-

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CELL LINE VALIDATION SHEET					
DRB1*15:01					
				Well	
					7
				Prod. No.	201550203
IHCW cell line			DRB1		
1	9001	SA	*01:01		-
2	9280	LK707	*15:02	*04:05	+
3	9011	E4181324	*15:02		+
4	9275	GU373	*03:01		-
5	9009	KAS011	*16:01		-
6	9353	SM	*04:07	*08:03	-
7	9020	QBL	*03:01		-
8	9025	DEU	*04:01		-
9	9026	YAR	*04:02		-
10	9107	LKT3	*04:05		-
11	9051	PITOUT	*07:01		-
12	9052	DBB	*07:01		-
13	9004	JESTHOM	*01:01		-
14	9071	OLGA	*08:02		-
15	9075	DKB	*09:01		-
16	9037	SWEIG007	*11:01		-
17	9282	CTM3953540	*03:01	*13:01	-
18	9257	32367	*09:01	*11:01	-
19	9038	BM16	*12:01		-
20	9059	SLE005	*13:02		-
21	9064	AMALA	*14:02		-
22	9056	KOSE	*13:02	*14:54	-
23	9124	IHL	*08:03	*14:14	-
24	9035	JBUSH	*11:01		-
25	9049	IBW9	*07:01		-
26	9285	WT49	*03:01		-
27	9191	CH1007	*04:05	*10:01	-
28	9320	BEL5GB	*04:16	*07:01	-
29	9050	MOU	*07:01		-
30	9021	RSH	*03:02		-
31	9019	DUCAF	*03:01		-
32	9297	HAG	*13:03		-
33	9098	MT14B	*04:04		-
34	9104	DHIF	*11:01		-
35	9302	SSTO	*04:03		-
36	9024	KT17	*04:03	*04:06	-
37	9065	HHKB	*13:01		-
38	9099	LZL	*14:02		-
39	9315	CML	*03:01	*04:01	-
40	9134	WHONP199	*07:01	*09:01	-
41	9055	H0301	*13:02		-
42	9066	TAB089	*08:03		-
43	9076	T7526	*09:01		-
44	9057	TEM	*14:01		-
45	9239	SHJO	*07:01		-
46	9013	SCHU	*15:01		+
47	9045	TUBO	*11:04	*12:01	-
48	9303	TER-ND	*01:03		-

101.901-24 – including *Taq* polymerase, IFU-01

101.901-24u – without *Taq* polymerase, IFU-02

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“Instructions for Use” (IFU)

Lot No.: **47X**

Lot-specific information

¹The provided cell line HLA specificities are retrieved from the <http://www.ihwg.org/hla> web site. The specificity of an individual cell line may thus be subject to change.

²The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs.

In primer solutions 5 and 7 one respective two 3'-primer was not possible to test, and in primer solution 7 two 5'-primers were not possible to test.

Additional 3'-primers in primer mix 7 were tested by separately adding one 5'-primer.

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Lot No.: **47X**

Lot-specific information

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