

101.211-24 – including *Taq* pol., IFU-01
101.211-24u – without *Taq* pol., IFU-02

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“Instructions for Use” (IFU)

Lot No.: **59X**

Lot-specific information
Olerup SSP® DQB1*05

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Product number:	101.211-24 – including <i>Taq</i> polymerase 101.211-24u – without <i>Taq</i> polymerase
Lot number:	59X
Expiry date:	2017-July-01
Number of tests:	24
Number of wells per test:	23+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. 59X

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*05 LOT (37V)

A well containing Negative Control primer pairs has been added.

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

The DQB1*05 primer set, specificity and interpretation tables have been updated for the HLA-DQB1 alleles described since the previous *Olerup SSP®* DQB1*05 lot was made (**Lot No. 37V**). The kit design is based on IMGT/HLA database 3.14.0.

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.

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

Well	5'-primer	3'-primer	rationale
7	Moved	Moved	Primer pair moved to well 11 for decreased tendency of primer oligomer formation.
11	Added	-	5'-primer added from well 7.
15	-	Modified	Modified 3'-primer for improved HLA-specific amplification.
23	-	Modified	Modified 3'-primer for improved HLA-specific amplification.
24	-	-	Updated negative control.

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Well **24** 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.

101.211-24 – including *Taq* pol., IFU-01
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PRODUCT DESCRIPTION

DQB1*05 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DQB1*05:01 to DQB1*05:47 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 24 PCR reactions in a 24 well 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	NC

The 24 well cut PCR plate is marked with 'DQ5' in silver gray ink.

Well No. 1 is marked with the Lot No. '59X'.

Wells 1 to 23 – DQB1*05 high resolution primers.

Well 24 – Negative Control (NC).

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 24 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

Due to the sharing of sequence motifs between DQB1 alleles, non-DQB1*05 alleles will be amplified by primer mixes 11, 12, 16, 17 and 22.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the DQB1*05 alleles, i.e. **DQB1*05:01 to DQB1*05:47**, recognized by the HLA Nomenclature Committee in October 2013^{1,2} will give rise to unique amplification patterns by the primers in the DQB1*05 subtyping kit.

The DQB1*05 subtyping kit cannot distinguish the silent mutations in the DQB1*05:01:01:01-05:01:08, the DQB1*05:02:01 and 05:02:03-05:02:07 alleles, the DQB1*05:03:01:01-05:03:10 alleles or the DQB1*05:05:01-05:05:02.

¹DQB1 alleles listed on the IMGT/HLA web page 2013-October-11, release 3.14.0, www.ebi.ac.uk/imgt/hla.

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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>.

The following DQB1*05 alleles can be distinguished by the different sizes of the specific PCR product:

Alleles	Primer mix
DQB1*05:27, 05:29	8

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DQB1*05 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

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DQB1*05 SSP subtyping

Specificities and sizes of the PCR products of the 23+1 primer mixes used for DQB1*05 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DQB1*05 alleles ³	Other amplified DQB1 alleles ⁴
1	225 bp	515 bp	*05:01:01:01-05:20, 05:22-05:33, 05:35-05:43, 05:45-05:47	
2	135 bp	430 bp	*05:01:01:01-05:01:09, 05:07, 05:11:01-05:12, 05:18, 05:20, 05:22, 05:25, 05:27, 05:29-05:32, 05:44-05:45	
3^{5,6}	120 bp	430 bp	*05:02:01-05:02:07, 05:05:01-05:05:02, 05:14, 05:17, 05:19, 05:26, 05:33-05:37, 05:46-05:47	
4^{5,6}	100 bp	515 bp	*05:01:09, 05:02:02, 05:03:01:01-05:03:10, 05:06:01, 05:08-05:10, 05:13, 05:15-05:16, 05:24, 05:28, 05:38-05:42	
5⁵	120 bp 185 bp	430 bp	*05:04 *05:10	
6	185 bp	430 bp	*05:05:01-05:05:02, 05:11:01	
7⁵	185 bp	430 bp	*05:06:01-05:07	
8	135 bp 190 bp	430 bp	*05:27 *05:09, 05:29	
9	130 bp	430 bp	*05:08, 05:18, 05:36	
10⁵	115 bp 195 bp	430 bp	*05:23 *05:12	
11⁵	100 bp 150 bp	515 bp	*05:13, 05:32, 05:42, 05:45 *05:35	*03:21
12⁵	120 bp 150 bp 195 bp	430 bp	*05:25 *05:14 *05:41N	*06:103
13	145 bp 180 bp 220 bp	430 bp	*05:40 *05:15, 05:33 *05:34, 05:44	
14	145 bp 200 bp	430 bp	*05:40 *05:16	
15^{5,6}	105 bp 135 bp	430 bp	*05:17 *05:37	
16	155 bp 195 bp	430 bp	*05:20 *05:47	*06:105
17⁵	125 bp	430 bp	*05:25, 05:31, 05:46	*06:111
18	180 bp	430 bp	*05:24	
19⁷	200 bp 225 bp	430 bp	*05:28, 05:30 *05:21	
20⁵	110 bp 270 bp	430 bp	*05:32, 05:42 *05:38	

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21	135 bp	430 bp	*05:37	
	200 bp		*05:39	
	230 bp		*05:26	
22⁵	70 bp	430 bp	*05:43	*06:51:01, 06:66, 06:96, 06:99:01
23⁵	120 bp	430 bp	*05:19	
			*05:22	
24⁸	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 DQB1*05 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.

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 longer, 515 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 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. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

⁴Due to the sharing of sequence motifs between DQB1 alleles, non-DQB1*05 alleles will be amplified by primer mixes 11, 12, 16, 17 and 22.

⁵HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁶Primer mixes 3, 4 and 15 may have tendencies of unspecific amplifications.

⁷Primer mix 19 has a tendency to giving rise to primer oligomer formation.

⁸Primer mix 24 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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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	225	135	120	100	120	185	185	135	130	115	100	120
					185			190		195	150	150
												195
Length of int. pos. control ¹	515	430	430	515	430	430	430	430	430	430	515	430
5'-primer(s) ²	26(173) 5'-ggg 3'	26(173) 5'-ggg 3'	29(184) 5'-gAC 3'	29(184) 5'-gAC 3'	29(184) 5'-gAT 3'	38(210) 5'-gCg 3'	39(212) 5'-gCA 3'	36(205) 5'-Agg 3'	135(501) 5'-gAT 3'	26(173) 5'-ggg 3'	13(136) 5'-gCC 3'	30(185) 5'-ACC 3'
					135(500) 5'-TgA 3'	38(210) 5'-gCA 3'	40(216) 5'-TTg 3'	139(512) 5'-gAT 3'			132(493) 5'-TTT 3'	118(449) 5'-CTA 3'
											146(533) 5'-CCg 3'	133(494) 5'-TCA 3'
											150(547) 5'-ACA 3'	
3'-primer(s) ³	87(356) 5'-ggT 3'	57(266) 5'-CAA 3'	56(265) 5'-gCT 3'	47(237) 5'-CgA 3'	56(265) 5'-gCT 3'	86(353) 5'-ACg 3'	87(356) 5'-ggT 3'	86(353) 5'-ACg 3'	163(585) 5'-gTT 3'	51(248) 5'-gCT 3'	32(191) 5'-TAC 3'	57(266) 5'-CAA 3'
	87(356) 5'-ggT 3'			47(237) 5'-CgA 3'	182(642) 5'-ggT 3'			169(604) 5'-gAC 3'	167(596) 5'-CAT 3'	77(328) 5'-CAA 3'	169(604) 5'-gAC 3'	169(604) 5'-gAC 3'
				50(246) 5'-gTg 3'								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15	16	17	18	19	20	21	22	23
Length of spec. PCR product	145	145	105	155	125	180	200	110	135	70	120
	180	200	135	195			225	270	200		
	220								230		
Length of int. pos. control ¹	430	430	430	430	430	430	430	430	430	430	430
5'-primer(s) ²	29(184) 5'-gAC 3'	29(184) 5'-gAC 3'	135(501) 5'-gAT 3'	19(154) 5'-ACC 3'	30(185) 5'-ACC 3'	29(184) 5'-gAC 3'	25(170) 5'-gCA 3'	9(122) 5'-gTT 3'	23(166) 5'-gCT 3'	47(237) 5'-TAC 3'	26(173) 5'-ggg 3'
				135(500) 5'-TgA 3'	140(517) 5'-CCA 3'		30(187) 5'-ACC 3'	146(533) 5'-CCg 3'	33(195) 5'-AAG 3'		
							37(208) 5'-ACA 3'		135(501) 5'-gAT 3'		
3'-primer(s) ³	65(290) 5'-CCC 3'	65(290) 5'-CCC 3'	157(566) 5'-ggg 3'	57(266) 5'-CAA 3'	57(266) 5'-CAA 3'	76(323) 5'-TgC 3'	87(356) 5'-ggT 3'	86(353) 5'-ACg 3'	87(356) 5'-ggT 3'	56(265) 5'-gTC 3'	49(242) 5'-CCT 3'
	74(317) 5'-CCA 3'	82(341) 5'-AgC 3'	166(595) 5'-ACA 3'	185(652) 5'-CAT 3'	169(604) 5'-gAC 3'			169(604) 5'-gAC 3'	166(595) 5'-ACA 3'		54(257) 5'-gCT 3'
	80(335) 5'-gTT 3'										
	88(361) 5'-CCT 3'										
Well No.	13	14	15	16	17	18	19	20	21	22	23

¹The 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 longer, 515 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.



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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										
DQB1*05 SSP subtyping kit										
					Well ²					
					17	18	19	20	21	22 23
				Production No.	201330817	201330818	201330819	201444220	201330821	201444222 201444223
	IHWC cell line ¹		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		-	-	-	-	-	-

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.

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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

No DNAs carrying the alleles to be amplified by primer solutions 5 to 23 were available. The specificities of the primers in primer solutions 5, 6, 8, 11, 16 and 20 to 22 were tested by separately adding one 5'-primer, respectively one 3'-primer. In primer solutions 7, 12, 17 and 19 it was only possible to test the 3'-primer, the 5'-primers were not possible to test. In primer solutions 9, 10, 13 to 15, 18 and 23 it was only possible to test the 5'-primer, the 3'-primers were not possible to test.

In primer solutions 8, 11, 16, 20 and 21 one, two or three 5'-primers were not possible to test, and in primer solutions 1, 4, 5, 11, 16 and 21 one or two 3'-primers were not possible to test.

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