101.201-48/12– including *Taq* pol., IFU-01
101.201-48u/12u– without *Taq* pol., IFU-02

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"Instructions for Use" (IFU)

Lot No.: 68V **Lot-specific information**

Olerup SSP® DQ low resolution

Product number:	**101.201-48/12 - including *Taq* pol.** **101.201-48u/12u - without *Taq* pol.**
Lot number:	**68V**
Expiry date:	**2016-October-01**
Number of tests:	**48 tests – Product No. 101.201-48/48u** **12 tests – Product No. 101.201-12/12u**
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. 68V.

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

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® DQ LOW RESOLUTION LOT (11S)

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

The 8 well cut PCR plate is marked with '68V' in silver/gray ink.

The DQ low resolution specificity and interpretation tables have been updated for the HLA-DQB1 alleles described since the previous *Olerup* SSP® DQ low resolution lot was made **(Lot No 11S)**.

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.

Lot No.: 68V **Lot-specific information**

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	Added	Added	5'-primer added for the DQB1*05:21 allele, 3'-primers added for the DQB1*05:03:10 and DQB1*05:34 alleles
2	Added	Added	Primer pair added for the DQB1*06:04:06 and 06:67 alleles, exchanged control primer pair.
3	Added	-	5'-primer added for the DQB1*02:25 allele.
4	-	Added	3'-primer added for the DQB1*03:01:08 allele.
5	-	Added	Strength of control band has been optimized, 3'-primer added for the DQB1*03:02:10 allele.
6	-	-	Strength of control band has been optimized
7	Exchanged	Added	5'-primers exchanged, 3'-primer added for the DQB1*04:09 and DQB1*03:01:08 alleles.
8	Added, modified	-	Strength of control band has been optimized, 5'-primer added for the DQB1*04:01:03 allele, 5'-primer modified for improved specificity.

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Lot No.: 68V **Lot-specific information**

PRODUCT DESCRIPTION

DQ low resolution SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for grouping the DQB1 alleles into the serological groups DQ2 to DQ9.

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 cut PCR plate is marked with '68V' in silver/gray ink.
Well No. 1 is marked with the Lot No. '68V'.
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 alleles will be amplified by the DQ low resolution typing kit. Thus, the interpretation of DQ low resolution typings is not influenced the DQB2 and DQB3 genes.

UNIQUELY IDENTIFIED ALLELES

All the DQB1 alleles, i.e. **DQB1*05:01 to 05:47, DQB1*06:01:01 to 06:119, DQB1*02:01:01 to 02:33, DQB1*03:01:01:01 to 03:100 and DQB1*04*01:01 to 04:17**, recognized by the HLA Nomenclature Committee in October 2013[1,2] will be amplified by the primers in the DQ low resolution SSP kit. The DQB1 alleles will be grouped into their corresponding serological specificities, i.e.:

DQ5(1) = DQB1*05:01-05:05[3]
DQ6(1) = DQB1*06:01-06:33[3]
DQ2 = DQB1*02:01-02:05
DQ3 = DQB1*03:01-03:20[3]
DQ4 = DQB1*04:01-04:02[3]

Lot No.: 68V **Lot-specific information**

The DQ3 alleles may be further subdivided into the DQ3, DQ7, DQ8 and DQ9 based upon serology and expert assignment. Thus;

DQ3	=	DQB1*03:06, 03:10, 03:14
DQ7	=	DQB1*03:01:01-03:01:03, 03:04, 03:09, 03:13, 03:16, 03:19
DQ8	=	DQB1*03:02:01, 03:05:01, 03:07, 03:08, 03:11, 03:18
DQ9	=	DQB1*03:03:02, 03:12, 03:15, 03:17, 03:20

[1]HLA-DQB1 alleles listed on the IMGT/HLA web page 2013-October-11, release 3.14.0, www.ebi.ac.uk/imgt/hla.
[2]Alleles 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.
[3]The serological split of the DQB1*05:05 to 05:47, DQB1*06:06 to 06:07, 06:10, 06:13, 06:15 to 06:24 and 06:27 to 06:119, the DQB1*02:04 to 02:33 the DQB1*03:02:02 to 03:02:04, 03:03:03, 03:05:02, 03:07 to 03:09 and 03:11 to 03:100 and the DQB1*04:0301 to 04:17 alleles is not known. The grouping of not serologically defined alleles is taken from the expert-assigned serological grouping in Tissue Antigens (2009) 73:95-170.

101.201-48/12– including *Taq* pol., IFU-01
101.201-48u/12u– without *Taq* pol., IFU-02

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Lot No.: 68V

Lot-specific information

SPECIFICITY TABLE

DQ low resolution SSP typing

Specificities and sizes of the PCR products of the 8 primer mixes used for DQ low resolution SSP typing

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	DQ serology[3]	Amplified DQB1 alleles[4]
1	225 bp	**515 bp**	5	*05:01:01:01-05:47
2[6]	220 bp, 275 bp	**515 bp**	1, 5, 6	*06:01:01-06:119
3	210 bp	430 bp	2	*02:01:01-02:33
4	220 bp	**515 bp**	3, 7	*03:01:01:01-03:01:21, 03:04, 03:09-03:10:01, 03:13-03:14:02, 03:16, 03:19, 03:21-03:22, 03:24, 03:27-03:29, 03:35-03:36, 03:42, 03:44, 03:46-03:60, 03:69, 03:71, 03:73, 03:75-03:77, 03:80, 03:82-03:84N, 03:92-03:94
5[5]	130 bp	**515 bp**	6, 8	*03:02:01-03:02:12, 03:05:01-03:05:04, 03:07-03:08, 03:11, 03:18, 03:32, 03:37, 03:45, 03:61, 03:63-03:64, 03:66N-03:68, 03:70, 03:85, 06:29
6[5,7]	135 bp	**515 bp**	2, 3, 4, 9	*02:03, 03:03:02:01-03:03:10, 03:06, 03:12, 03:15, 03:20, 03:25-03:26, 03:30-03:31, 03:33-03:34, 03:38-03:41, 03:43, 03:65, 03:74, 03:79, 03:86-03:91Q, 03:95N-03:99Q, 04:03:01-04:03:02, 06:03:10, 06:51:01, 06:66, 06:96
7[5,6]	85 bp, 185 bp	**515 bp**	3, 7, 8, 9	*03:01:01:01-03:100, 04:01:03
8[5,6]	160 bp, 205 bp	430 bp	4	*04:01:01-04:17

[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 DQ low resolution 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 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.

101.201-48/12– including *Taq* pol., IFU-01
101.201-48u/12u– without *Taq* pol., IFU-02

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Lot No.: 68V **Lot-specific information**

[2]The 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. In the presence of a specific amplification the intensity of the control band often decreases.

[3]The serological split of the DQB1*05:05 to 05:47, DQB1*06:06 to 06:07, 06:10, 06:13, 06:15 to 06:24 and 06:27 to 06:119, the DQB1*02:04 to 02:33 the DQB1*03:02:02 to 03:02:04, 03:03:03, 03:05:02, 03:07 to 03:09 and 03:11 to 03:100 and the DQB1*04:03:01 to 04:17 alleles is not known. The grouping of not serologically defined alleles is taken from the expert-assigned serological grouping in Tissue Antigens (2009) 73:95-170.

[4]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. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

[5]Primer mixes 5 to 8 may give a lower yield of HLA-specific PCR products than the other DQ low resolution primer mixes.

[6]The primer pairs in wells 2, 7 and 8 will in some samples give rise to two HLA-specific PCR fragments.

[7]Primer mix 6 may have a tendency to giving rise to primer oligomer formation.

'ser', serological HLA specificity

101.201-48/12– including *Taq* pol., IFU-01
101.201-48u/12u– without *Taq* pol., IFU-02

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Lot No.: 68V **Lot-specific information**

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8
Length of spec.	225	220	210	220	130	135	85	160
PCR product		275					185	205
Length of int.	515	515	430	515	515	515	515	430
pos. control[1]								
5'-primer(s)[2]	25(170)	9(122)	29(184)	26(173)	28(179)	26(173)	38(210)	23(164)
	5' -gCA 3'	5' -gTT 3'	5' -gAg 3'	5' -TTA 3'	5' -gAC 3'	5' -TCT 3'	5' -gCA 3'	5' -gCT 3'
	26(173)	24(169)	30(185)		28(179)		71(309)	38(210)
	5' -ggg 3'	5' -TgT 3'	5' -AAg 3'		5' -gAC 3'		5' -ACC 3'	5' -gCg 3'
		26(173)					71(309)	
		5' -TTA 3'					5' -ACC 3'	
		26(173)						
		5' -TCT 3'						
3'-primer(s)[3]	87(356)	86(353)	86(353)	86(353)	57(266)	57(266)	86(353)	77(327)
	5' -ggT 3'	5' -ACg 3'	5' -gCT 3'	5' -gCT 3'	5' -Cgg 3'	5' -CgT 3'	5' -gCT 3'	5' -ACg 3'
	87(356)	86(353)		86(354)	57(266)		86(354)	
	5' -ggT 3'	5' -ACC 3'		5' -AgT 3'	5' -CAg 3'		5' -AgT 3'	
	88(361)	86(354)						
	5' -CCT 3'	5' -TAT 3'						
Well No.	1	2	3	4	5	6	7	8

[1]The 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 88 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.

[2]The 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.

[3]The 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.

101.201-48/12– including *Taq* pol., IFU-01
101.201-48u/12u– without *Taq* pol., IFU-02

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"Instructions for Use" (IFU)

Lot No.: 68V **Lot-specific information**

CELL LINE VALIDATION SHEET

DQ low resolution primer set[2]

					Well							
					1	2	3	4	5	6	7	8
				Production No.	201434401	201434402	201434403	201331704	201331705	201331706	201331707	201434408
	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	CTM 3953540	*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		+	-	-	-	-	-	-	-

101.201-48/12– including *Taq* pol., IFU-01
101.201-48u/12u– without *Taq* pol., IFU-02

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"Instructions for Use" (IFU)

Lot No.: 68V **Lot-specific information**

[1]The 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.

[2]The 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 1, 2 and 3 one 5'-primer was not possible to test, and in primer solutions 1, 2, 4, 5 and 7 one or two 3'-primers were not possible to test.

Additional 5'-primers in primer solutions 2 and 7 were tested by separately adding another 3'-primer.

101.201-48/12– including *Taq* pol., IFU-01
101.201-48u/12u– without *Taq* pol., IFU-02

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Lot No.: 68V **Lot-specific information**

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Lot No.: 68V **Lot-specific information**

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