101.814-12 – including *Taq* polymerase, IFU-01
101.814-12u – without *Taq* polymerase, IFU-02

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Lot No.: 7D0 **Lot-specific information**

Olerup SSP® DRB1*04 Add-on

Product number:	**101.814-12 – including *Taq* pol.** **101.814-12u – without *Taq* pol.**
Lot number:	**7D0**
Expiry date:	**2018-10-01**
Number of tests:	**12**
Number of wells per test:	**3+1**
Storage - pre-aliquoted primers:	**dark at -20ºC**
- PCR Master Mix:	**-20ºC**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 7D0.

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

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® DRB1*04 ADD-ON LOT (68X)

A well containing Negative Control primer pairs has been added.

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

The DRB1*04 Add-on specificity and interpretation tables have been updated for the DRB1 alleles described since the previous *Olerup* SSP® DRB1*04 Add-on lot was made **(Lot No. 68X)**. The kit design is based on IMGT/HLA database 3.22.0.

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.

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

Well	5'-primer	3'-primer	rationale
4	-	-	Updated negative control.

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Well **4** 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'-primer[1]	**164**	**340**	**440**	**45**	**45**	**43**	**36**
	5'-CAC3'	**5'-Agg3'**	**5'-TTA3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-TAC3'**
							36
							5'-TAT3'
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**	**47**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**	**5'-ACA3'**
							48
							5'-gCA3'
							48
							5'-gCC3'
							52
							5'-TgT3'
A*	**+**	**+**	**+**				
B*	**+**	**+**	**+**				
C*	**+**	**+**	**+**				
DRB1				**+**	**+**		
DRB3				**+**	**+**		
DRB5				**+**			
DQB1					**+**		
DPB1						**+**	
DQA1							**+**

[1]The 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 codonnumbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.
[2]The 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.

101.814-12 – including *Taq* polymerase, IFU-01
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Lot No.: 7D0 **Lot-specific information**

PRODUCT DESCRIPTION

DRB1*04 Add-on SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for separating the DRB1*04:92 from the DRB1*04:07 alleles.
The primer set also resolves the SBT heterozygous ambiguities:
DRB1*04:02:01, 04:03:01 = DRB1*04:37, 04:88
DRB1*04:02:01, 04:04:01 = DRB1*04:37, 04:56

PLATE LAYOUT

Each test consists of 4 PCR reactions in an 8 well cut PCR plate. Wells 5 to 8 are empty.

1	**2**	**3**	**NC**	**empty**	**empty**	**empty**	**empty**

The 8 well cut PCR plate is marked with '7D0' in silver/gray ink.
Well No. 1 is marked with the Lot No. '7D0'.
Wells 1 to 3 – DRB1*04 Add-on high resolution primers.
Well 4 – 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 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

The interpretation of DRB1*04 Add-on PCR-SSP subtypings will be influenced by many other DRB1*04 alleles.
For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

The DRB1*04:07 and DRB1*04:92 alleles give different patterns in the DRB1*04 Add-on subtyping kit[1,2].
The primer set also resolves the SBT heterozygous ambiguities:
DRB1*04:02:01, 04:03:01 = DRB1*04:37, 04:88
DRB1*04:02:01, 04:04:01 = DRB1*04:37, 04:56

The DRB1*04 Add-on subtyping kit cannot distinguish the following silent mutations: the DRB1*04:02:01-04:02:05, the DRB1*04:03:01-04:03:11 and the DRB1*04:07:01-04:07:05 alleles.

101.814-12 – including *Taq* polymerase, IFU-01
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[1]Based on DRB alleles listed on the IMGT/HLA web page 2015-October-10, release 3.22.0, 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.

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Lot No.: 7D0 **Lot-specific information**

SPECIFICITY TABLE

DRB1*04 Add-on SSP subtyping

Specificities and sizes of the PCR products of the 3+1 primer mixes used for DRB1*04 Add-on SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified DRB1*04 alleles[3,4]
1	170 bp	**515 bp**	*04:01:01-04:01:15, 04:02:01-04:04:06, 04:04:08-04:04:10, 04:06:01-04:08:02, 04:08:04, 04:11:03, 04:13-04:14, 04:16, 04:18-04:23, 04:25, 04:26[w], 04:27, 04:31-04:33, 04:35-04:44, 04:46-04:47, 04:49-04:56:02, 04:58-04:60, 04:63, 04:65, 04:68, 04:70-04:76, 04:78-04:79, 04:85, 04:88, 04:92-04:98:02, 04:100-04:102, 04:105:01-04:105:02, 04:109-04:115, 04:117-04:124, 04:127-04:130, 04:132-04:135, 04:139-04:144, 04:148-04:151, 04:153, 04:155-04:159, 04:161, 04:163-04:168, 04:171-04:172, 04:174-04:177, 04:179-04:190, 04:192-04:200
2[5]	75 bp	430 bp	*04:92
3	200 bp	430 bp	*04:37, 04:58, 04:132
4[6]	-	-	**Negative Control**

[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 DRB1*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 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.

[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.

[3]For several 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.

[4]Due to the sharing of sequence motifs many DRB1*04 alleles are amplified by primer mix 1.

101.814-12 – including *Taq* polymerase, IFU-01
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[5]HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

[6]Primer mix 4 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.

'w', might be weakly amplified.

101.814-12 – including *Taq* polymerase, IFU-01
101.814-12u – without *Taq* polymerase, IFU-02

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Lot No.: 7D0 **Lot-specific information**

PRIMER SPECIFICATION

Well No.	1	2	3
Length of spec.	170	75	200
PCR product			
Length of int.	515	430	430
pos. control[1]			
5'-primer(s)[2]	13(125)	196(674)	13(125)
	5' -ACA 3'	5' -ACA 3'	5' -ACA 3'
3'-primer(s)[3]	56(256)	206(706)	66(286)
	5' -ATC 3'	5' -CAT 3'	5' -gAg 3'
	56(256)		
	5' -ATC 3'		
	58(261)		
	5' -TCA 3'		
Well No.	1	2	3

[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 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.

[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.

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Lot No.: 7D0 **Lot-specific information**

CELL LINE VALIDATION SHEET

DRB1*04 Add-on SSP subtyping kit[2]

					Well		
					1	2	3
				Prod. No.:	201320201	201320202	201320203
	IHWC cell line[1]		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		-	-	-

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[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.

No DNAs carrying the alleles to be amplified by primer solutions 2 and 3 were available. The specificity of the primers in primer solution 3 was tested by separately adding additional 5'-primers respectively 3'-primers. In primer solution 2 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solution 1 one of the 3'-primers could not be tested.

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Lot No.: 7D0 **Lot-specific information**

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