

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

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **50S**

Lot-specific information

Olerup SSP[®] DRB1*04 Add-on

Product number:	101.814-12– including <i>Taq</i> pol. 101.814-12u– without <i>Taq</i> pol.
Lot number:	50S
Expiry date:	2015-December-01
Number of tests:	12
Number of wells per test:	3
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. 50S.

The Lot-specific information for DRB1*04 Add-on including and without *Taq* polymerase is described in one common Product Insert.

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. 30N**).

The DRB1*04 Add-on primer set is unchanged compared to the previous *Olerup SSP[®]* DRB1*04 Add-on (**Lot No. 30N**).

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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 3 PCR reactions in an 8 well cut PCR plate. Wells 4 to 8 are empty.

1	2	3	empty	empty	empty	empty	empty
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The 8 well cut PCR plate is marked with '50S' in silver/gray ink.

Well No. 1 is marked with the Lot No. '50S'.

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.

UNIQUELY IDENTIFIED ALLELES

The DRB1*04:07 and DRB1*04:92 alleles give different patterns in the DRB1*04 Add-on subtyping kit¹.

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*04:02:01-04:02:03, the DRB1*04:03:01-04:03:08 and the DRB1*04:07:01-04:07:04 alleles.

¹Based on DRB alleles listed on the IMGT/HLA web page 2013-April-17, release 3.12.0, www.ebi.ac.uk/imgt/hla.

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SPECIFICITY TABLE

DRB1*04 Add-on SSP subtyping

Specificities and sizes of the PCR products of the 3 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	*04:01:01-04:04:06, 04:04:08, 04:06:01-04:08:02, 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, 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, 04:155-04:158N
2⁵	75 bp	430 bp	*04:92
3	200 bp	430 bp	*04:37, 04:58, 04:132

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*14 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 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.

² The two different control primer pairs give rise to an internal positive control band of either 430 or 515 base pairs.

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 DRB1*04 Add-on subtyping.

In the presence of a specific amplification the intensity of the control band often decreases.

³ For several DRB 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 and that unknown sequences of codons 87 to 92 are identical with the DRB1*01:01 consensus sequence.

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

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

‘w’, might be weakly amplified.

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INTERPRETATION TABLE			
DRB1*04 Add-on SSP typing			
	Well		
	1	2	3
Length of spec.	170	75	200
PCR product			
Length of int.	515	430	430
pos. control¹			
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
DRB1 allele			
*04:01:01-04:04:06, 04:04:08, 04:06:01-04:08:02, 04:11:03, 04:13-04:14, 04:16, 04:18-04:23, 04:25, 04:27, 04:31-04:33, 04:35-04:36, 04:38-04:44, 04:46-04:47, 04:49-04:56, 04:59-04:60, 04:63, 04:65, 04:68, 04:70-04:76, 04:78-04:79, 04:85, 04:88, 04:93-04:98:02, 04:100-04:102, 04:105:01-04:105:02, 04:109-04:115, 04:117-04:124, 04:127-04:130, 04:133-04:135, 04:139-04:144, 04:148-04:151, 04:153, 04:155-04:158N	1		
*04:26	w		
*04:37, 04:58, 04:132	1		3
*04:92	1	2	
DRB1 allele			
Well No.	1	2	3

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to an internal positive control band of either 430 or 515 base pairs.

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 DRB1*04 Add-on subtyping.

²The codon, and in parenthesis the nucleotide, in the 2nd and 4th 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.

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Lot No.: 50S**Lot-specific information**

³The codon, and in parenthesis the nucleotide, in the 2nd and 4th 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.

'w', might be weakly amplified.

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CELL LINE VALIDATION SHEET						
DRB1*04 Add-on SSP subtyping kit						
				Well		
				1	2	3
				201320201	201320202	201320203
				Prod. No.:		
IHWC 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		-	-	-

101.814-12 – including *Taq* polymerase, IFU-01101.814-12u – without *Taq* polymerase, IFU-02Visit www.olerup-ssp.com for

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CERTIFICATE OF ANALYSIS

Olerup SSP® DRB1*04 Add-on SSP

Product number: 101.814-12 – including *Taq* pol.101.814-12u – without *Taq* pol.

Lot number: 50S

Expiry date: 2015-December-01

Number of tests: 12

Number of wells per test: 3

Well specifications:

Well No.	Production No.
1	2013-202-01
2	2013-202-02
3	2013-202-03

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 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 mix 1 one of the 3'-primers could not be tested.

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

Date of approval: 2013-August-16

Approved by:

Production Quality Control

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Declaration of Conformity

Product name: *Olerup* SSP® DRB1*04 Add-on

Product number: 101.814-12/12u

Lot number: 50S

Intended use: DRB1*04 high 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 13485:2012, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, conformity assessed using Annex IV, 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.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom.
(Notified Body number: 0088.)

Stockholm, Sweden
2013-August-16

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

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