101.814-12 – including *Taq* polymerase, IFU-01 Rev. No. 03
101. 815-12u – without *Taq* polymerase, IFU-02 Rev. No. 03

Visit www.olerup-ssp.com for **"Instructions for Use" (IFU)**

Lot No.: 30N **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:	**30N**
Expiry date:	**2014-August-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. 30N.

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

101.814-12 – including *Taq* polymerase, IFU-01 Rev. No. 03
101. 815-12u – without *Taq* polymerase, IFU-02 Rev. No. 03

Visit www.olerup-ssp.com for **"Instructions for Use" (IFU)**

Lot No.: 30N **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 3 PCR reactions in an 8 well cut PCR plate. Wells 4 to 8 are empty.

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

The 8 well cut PCR plate is marked with '30N' in silver/gray ink.
Well No. 1 is marked with the Lot No. '30N'.
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 kit[1].
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*04:03:01-04:03:07 and the DRB1*04:07:01-04:07:04 alleles.

[1]Based on DRB alleles listed on the IMGT/HLA web page 2012-January-12, release 3.7.0, www.ebi.ac.uk/imgt/hla.

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, 04:06:01-04:08:02, 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
2[5]	75 bp	430 bp	*04:92
3	200 bp	430 bp	*04:37, 04:58

[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 specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective lengths of the specific PCR product(s) of the alleles amplified by these primer mixes are given.
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 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 only partial nucleotide sequences from the second exon are available. In these instances it is not known whether some of the primers of the SSP set are completely matched with the target sequences or not. We assume that unknown sequences in the first hyperpolymorphic region of the second exon of DRB alleles are conserved within allelic groups and that unknown sequences of codons 87 to 92 are identical with the DRB1*01:01 consensus sequence.
[4]Due to the sharing of sequence motifs many DRB1*04 alleles are amplified by primer mix 1.
[5]Short specific PCR fragments have a lower intensity than longer PCR bands.
'w', might be weakly amplified.

101.814-12 – including *Taq* polymerase, IFU-01 Rev. No. 03
101. 815-12u – without *Taq* polymerase, IFU-02 Rev. No. 03

Visit www.olerup-ssp.com for **"Instructions for Use" (IFU)**

Lot No.: 30N **Lot-specific information**

INTERPRETATION TABLE

DRB1*04 Add-on SSP typing

	Well		
	1	**2**	**3**
Length of spec. PCR product	**170**	**75**	**200**
Length of int. pos. control[1]	**515**	**430**	**430**
5'-primer(s)[2]	**13(125)** 5' -ACA 3'	**196(674)** 5' -ACA 3'	**13(125)** 5' -ACA 3'
3'-primer(s)[3]	**57(256)** 5' -ATC 3'	**207(706)** 5' -CAT 3'	**67(286)** 5' -gAg 3'
	57(256) 5' -ATC 3'		
	58(261) 5' -TCA 3'		
Well No.	**1**	**2**	**3**
DRB1 allele			
***04:01:01-04:04:06, 04:06:01-04:08:02, 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**	**1**		
***04:26**	**w**		
***04:37, 04:58**	**1**		**3**
***04:92**	**1**	**2**	
DRB1 allele			
Well No.	**1**	**2**	**3**

[1]The 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.
[2]The 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.
[3]The 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.

101.814-12 – including *Taq* polymerase, IFU-01 Rev. No. 03
101. 815-12u – without *Taq* polymerase, IFU-02 Rev. No. 03

Visit www.olerup-ssp.com for **"Instructions for Use" (IFU)**

Lot No.: 30N **Lot-specific information**

CELL LINE VALIDATION SHEET

DRB1*04 Add-on SSP subtyping kit

					Well		
					1	2	3
				Prod. No.:	201296801	201296802	201296803
	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		-	-	-

Lot No.: 30N **Lot-specific information**

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: **30N**
Expiry date: **2014-August-01**
Number of tests: **12**
Number of wells per test: **3**

Well specifications:

Well No.	Production No.
1	2012-968-01
2	2012-968-02
3	2012-968-03

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: 2012-March-09

Approved by:

Production Quality Control

Lot No.: 30N **Lot-specific information**

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*04 Add-on
Product number: 101.814-12/12u
Lot number: 30N

Intended use: DRB1*12 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 13485:2003, 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
2012-July-04

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

101.814-12 – including *Taq* polymerase, IFU-01 Rev. No. 03
101. 815-12u – without *Taq* polymerase, IFU-02 Rev. No. 03

Visit www.olerup-ssp.com for **"Instructions for Use" (IFU)**

Lot No.: 30N **Lot-specific information**

ADDRESSES:

Manufacturer:
Olerup SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.
Tel: +46-8-717 88 27
Fax: +46-8-717 88 18
E-mail: info-ssp@olerup.com
Web page: http://www.olerup-ssp.com

Distributed by:
Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.
Tel: +43-1-710 15 00
Fax: +43-1-710 15 00 10
E-mail: support-at@olerup.com
Web page: http://www.olerup.com

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382
Tel: 1-877-OLERUP1
Fax: 610-344-7989
E-mail: info.us@olerup.com
Web page: http://www.olerup.com

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.