Olerup SSP® DRB1*15/16 low resolution

Product number:	**101.151-24 – including *Taq* polymerase**
Lot number:	**48E**
Expiry date:	**2010-May-01**
Number of tests:	**24**
Number of wells per test:	**2**
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. 48E.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® DRB1*15/16 LOW RESOLUTION LOT

The DRB1*15/16 low resolution specificity and interpretation tables have been updated for the DRB1 alleles described since the previous *Olerup* SSP® DRB1*15/16 low resolution lot was made **(Lot No. Y18)**.

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	Primer added for the DRB1*1527 allele.
2	Added	-	Primer added for the DRB1*1612 allele.

PRODUCT DESCRIPTION

DRB1*15/16 low resolution SSP

CONTENT
The primer set contains 5'- and 3'-primers for identifying the DRB1*15 and DRB1*16 group of alleles.

PLATE LAYOUT
Each test consists of 2 PCR reactions. 4 tests are aliquoted in each cut 8 well PCR plate.

1	**2**	**1**	**2**	**1**	**2**	**1**	**2**

The 8 well cut PCR plate is marked with 'DR2'.
Well No. 1 is marked with the Lot No. '48E'.
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 DRB1*15 and DRB1*16 alleles will be amplified by the DRB1*15/16 low resolution kit. Thus, the interpretation of DRB1*15/16 low resolution typings is not influenced by other groups of DRB1 alleles or other DRB genes.

UNIQUELY IDENTIFIED ALLELES
All the DRB1*15 and DRB1*16 alleles, i.e. **DRB1*1501 to DRB1*1527 and DRB1*1601 to DRB1*1613N**, recognized by the HLA Nomenclature Committee in April 2008[1] will be amplified by the primers in the DRB1*15/16 subtyping kit and grouped into the DRB1*15 and DRB1*16 groups, respectively.

[1]DRB1 alleles listed on the IMGT/HLA web page 2008-April-08, release 2.21.0, www.ebi.ac.uk/imgt/hla.

SPECIFICITY TABLE

DRB1*15/16 SSP low resolution

Specificities and sizes of the PCR products of the 2 primer mixes used for DRB1*15/16 SSP low resolution typing

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified DRB1*15/16 alleles[3]
1	215, 260 bp	**515 bp**	150101-1527
2	210 bp	430 bp	160101-160502, 1607-1613N

[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*15/16 low resolution SSP subtypings.
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 length 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 internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.
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*15/16 low resolution typing.
In the presence of a specific amplification the intensity of the control band often decreases.
[3]For several DRB alleles only partial second exon nucleotide sequences are 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 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*0101 consensus sequence.

INTERPRETATION TABLE

DRB1*15/16 low resolution typing

	Well		
	1	2	
Length of spec.	215	210	Length of spec.
PCR product	260		PCR product
Length of int.	515	430	Length of int.
pos. control[1]			pos. control[1]
5'-primer[2]	13	13	5'-primer[2]
	5'-Agg3'	5'-Agg3'	
		13	
		5'-AAg3'	
3'-primer(s)[3]	70	67	3'-primer(s)[3]
	5'-CTg3'	5'-gAA3'	
	71	67	
	5'-CCA3'	5'-gAg3'	
	86	72	
	5'-CCA3'	5'-gC g3'	
Well No.	1	2	Well No.
DRB1 allele[4]			DRB1 allele[4]
*150101-1527	1		*150101-1527
*160101-160502, 1607-1613N		2	*160101-160502, 1607-1613N
DRB1 allele[4]			DRB1 allele[4]
Well No.	1	2	Well No.

[1]The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.
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*15/16 low resolution typing.
[2]The codon, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given. 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. Empty spaces indicate codon boundaries.
[3]The codon, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. 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. Empty spaces indicate codon boundaries.
[4]The DRB1*1606 allele has been shown to be identical to DRB1*1605.

CELL LINE VALIDATION SHEET

DRB1*15/16 SSP low reolution

					Well	
					1	2
				Prod. No.:	200845201	200845202
	IHWC cell line		DRB1			
1	9001	SA	*0101		-	-
2	9280	LK707	*1502	*0405	+	-
3	9011	E4181324	*1502		+	-
4	9275	GU373	*0301		-	-
5	9009	KAS011	*1601		-	+
6	9353	SM	*0407	*0803	-	-
7	9020	QBL	*0301		-	-
8	9007	DEM	*0401	*1602	-	+
9	9026	YAR	*0402		-	-
10	9107	LKT3	*0405		-	-
11	9051	PITOUT	*0701		-	-
12	9052	DBB	*0701		-	-
13	9067	BTB	*0801		-	-
14	9071	OLGA	*0802		-	-
15	9075	DKB	*0901		-	-
16	9037	SWEIG007	*1101		-	-
17	9008	WILJON	*1501		+	-
18	9257	32367	*0901	*1101	-	-
19	9038	BM16	*1201		-	-
20	9059	SLE005	*1302		-	-
21	9064	AMALA	*1402		-	-
22	9056	KOSE	*1302	*1401	-	-
23	9124	IHL	*0803	*1414	-	-
24	9035	JBUSH	*1101		-	-
25	9049	IBW9	*0701		-	-
26	9285	WT49	*0301		-	-
27	9191	CH1007	*0405	*1001	-	-
28	9320	BEL5GB	*0416	*0701	-	-
29	9050	MOU	*0701		-	-
30	9021	RSH	*0302		-	-
31	9019	DUCAF	*0301		-	-
32	9297	HAG	*1303		-	-
33	9098	MT14B	*0404		-	-
34	9104	DHIF	*1101			-
35	9302	SSTO	*0403		-	-
36	9024	KT17	*0403	*0406	-	-
37	9065	HHKB	*1301		-	-
38	9099	LZL	*1402		-	-
39	9315	CML	*0301	*0401	-	-
40	9134	WHONP199	*0701	*0901	-	-
41	9055	H0301	*1302		-	-
42	9066	TAB089	*0803		-	-
43	9076	T7526	*0901		-	-
44	9057	TEM	*1401		-	-
45	9239	SHJO	*0701		-	-
46	9013	SCHU	*1501		+	-
47	9045	TUBO	*1104	*1201	-	-
48	9303	TER-ND	*0103		-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® DRB1*15/16 low resolution SSP

Product number: **101.151-24 – including *Taq* polymerase**
Lot number: **48E**
Expiry date: **2010-May-01**
Number of tests: **24**
Number of wells per test: **2**

Well specifications:

Well No.	Production No.
1	2008-452-01
2	2008-452-03

The specificity of each primer solution of the kit has been tested against 48 well characterized cell line DNAs.

Additional 3'-primers in primer solutions 1 and 2 were tested by separately adding one additional 3'-primer.

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

Date of approval: 2009-May-25

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*15/16 low resolution
Product number: 101.151-24
Lot number: 48E

Intended use: DRB1*15/16 low resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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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 9001:2000 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, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

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

Saltsjöbaden, Sweden
2009-May-25

Olle Olerup
Managing Director

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