

Lot No.: **02F**

Lot-specific information

***Olerup* SSP[®] HLA-B*59**

Product number: 101.554-06u – without *Taq* polymerase
Lot number: 02F
Expiry date: 2010-August-01
Number of tests: 6
Number of wells per test: 6
Storage - pre-aliquoted primers: dark at -20°C
- PCR Master Mix: -20°C

This Product Description is only valid for Lot No. 02F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP[®] HLA-B*59 LOT.

The HLA-B*59 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup* SSP[®] HLA-B*59 lot (**Lot No. Y44**).

Two wells have been added to the HLA-B*59 kit,
wells **5 and 6**.

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

Well	5'-primer	3'-primer	rationale
5	New	New	New primer pair to distinguish the B*5902 and B*5621 alleles.
6	New	New	New primer pair for the B*5903 allele.

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PRODUCT DESCRIPTION

HLA-B*59 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*5901 to B*5903 alleles.
subtyping.

PLATE LAYOUT

Each HLA-B*59 test consists of 6 PCR reactions in an 8 well cut PCR plate. Wells 7 and 8 are empty.

1	2	3	4	5	6	empty	empty
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The 8 well PCR plate is marked with 'B59' in silver/gray ink.

Well No. 1 is marked with the Lot No. '02F'.

The PCR plates are heat-sealed with a PCR-compatible foil.

Please note: When removing each 8 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

The interpretation of HLA-B*59 SSP subtypings will be influenced by the B*0765, B*0809, several B*13, three B*15, the B*2714, two B*35, four B*39, nine B*40, several B*41, the B*4204, four B*44, most B*45, three B*49, the B*50, most B*51, the B*52, most B*54, the B*55, several B*56, the B*5808, the B*650102, the B*7301, the B*78, the B*81, the B*82 and the B*8301 alleles when present on the other haplotype.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*59, i.e. **B*5901 to B*5903**, recognized by the HLA Nomenclature Committee in July 2008¹ will be amplified by the primers in the HLA-B*59 SSP kit.

¹HLA-B alleles listed on the IMGT/HLA web page 2008-July-11, release 2.22.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The three HLA-B*59 alleles can be combined in 6 homozygous and heterozygous combinations. All these genotypes give rise to unique amplification patterns.

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SPECIFICITY TABLE

HLA-B*59 SSP subtyping

Specificities and sizes of the PCR products of the 6 primer mixes used for HLA-B*59 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*59 alleles	Other amplified HLA-B alleles ³
1	430 bp	800 bp	5901-5903	5412, 5621
2⁴	105 bp	1070 bp	5901	130201-1303, 1308Q, 1309, 1314-1316, 1318, 1319, 1542, 3560, 4415, 4418, 4501, 4503-4508, 4611, 4901-4903, 5001, 5002, 5004, 5115, 5401-5403, 5405N, 5407, 5408N, 5410, 5412, 5413, 550101-5503, 5505, 5507, 5509-5512, 5515, 5516, 5518, 5519, 5521, 5522, 5524-5526, 5529-5531, 5601, 5607, 5608, 5613, 5614, 5616, 5617, 5619N, 5620, 55623
3⁴	105 bp	1070 bp	5902	0809, 1583, 2714, 40060101-400602, 4044, 4053, 4070, 4075, 4083, 4101, 4105-4107, 4204, 4420, 4447, 510101-5103, 5105, 5107-5112, 5114, 5116-5124, 5126-5136, 5138-5141N, 5143, 5144N, 5148-5155, 5157, 520101-5213, 5404, 5411, 5415, 5517, 5520, 5527, 5528, 560501-5606, 5615, 5621, 5808, 7801-7806
4	150 bp	1070 bp		5412
5	400 bp	1070 bp		0765, 3576, 5401-5406, 5408N-5415, 550101-5505, 5507-5517, 5519-5532, 5601-5613, 5615,

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				5616, 5618-5622, 670102, 8101, 8103- 8104N ² , 8201, 8202, 8301
6⁴	105 bp	1070 bp	5903	9537, 390601-390602, 3933, 3934, 4086, 4502, 511301-511302, 5214, 5622, 7301

¹Alleles 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 HLA-B*59 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 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 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.

²The 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 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number contains the primer pair giving rise to the shorter, 800 bp, internal positive control band.

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

³Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B*59 alleles will be amplified by primer mixes 1 to 6.

⁴Short specific PCR fragments are less intense and not as sharp as longer specific bands.

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INTERPRETATION TABLE						
HLA-B*59 SSP subtyping						
Amplification patterns of the B*5901-5903 alleles						
	Well					
	1	2	3	4	5	6
Length of spec.	430	105	105	150	400	105
PCR product						
Length of int.	800	1070	1070	1070	1070	1070
pos. control¹						
5'-primer²	48	357	357	206	41	357
	^{5'} -gCC ^{3'}	^{5'} -Tgg ^{3'}	^{5'} -Tgg ^{3'}	^{5'} -Agg ^{3'}	^{5'} -CTg ^{3'}	^{5'} -Tgg ^{3'}
3'-primer³	309	420	419	317	272	418
	^{5'} A _{TC} ^{3'}	^{5'} -gCT ^{3'}	^{5'} -CgT ^{3'}	^{5'} -ggA ^{3'}	^{5'} TgT ^{3'}	^{5'} CgA ^{3'}
Well No.	1	2	3	4	5	6
HLA-B allele						
*5901	1	2				
*5902	1		3			
*5903	1					6
*0756, 3576, 5406, 5409, 5414, 5504, 5508, 5514, 5523, 5532, 5602-5604, 5609-5612, 5618, 670102, 8101, 8201, 8202, 8301					5	
*0809, 1583, 2714, 40060101-400602, 4044, 4053, 4070, 4075, 4083, 4101, 4105-4107, 4204, 4420, 4447, 510101-5103, 5105, 5107-5112, 5114, 5116-512402, 5126-5136, 5138-5141N, 5143, 5144N, 5148-5155, 5157, 520101-5213, 5808, 7801-7806			3			
*130201-1303, 1308Q, 1309, 1314-1316, 1318, 1319, 1542, 3560, 4415, 4418, 4501, 4503-4508, 4611, 4901-4903, 5001, 5002, 5004, 5115, 5407, 5518, 5614, 5617, 5623		2				
*9537, 390601-390602, 3933, 3934, 4086, 4502, 511301-511302, 5214, 7301						6
*5401-5403, 5405N, 5408N, 5410, 5413, 550101-5503, 5505, 5507, 5509-5512, 5515, 5516, 5519, 5521, 5522, 5524-5526, 5529-5531, 5601, 5607, 5608, 5613, 5616, 5619N, 5620		2			5	
Well No.	1	2	3	4	5	6

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Length of spec.	430	105	105	150	400	105
PCR product						
Well No.	1	2	3	4	5	6
*5404, 5411, 5415, 5517, 5520, 5527, 5528, 560501-5606, 5615			3		5	
*5412	1	2		4	5	
*5513, 5622					5	6
*5621	1		3		5	
8103, 8104					?	
HLA-B allele						
Well No.	1	2	3	4	5	6

¹The 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 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band.

²The nucleotide position, in the 1st or 3rd exon, 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.

³The nucleotide position, in the 2nd or 3rd exon, 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.

'?', nucleotide sequence information for the primer matching region not known.

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CELL LINE VALIDATION SHEET										
HLA-B*59 SSP primer set										
				Prod. No.:	Well					
					1	2	3	4	5	6
					200624801	200624802	200624803	200739604	200850505	200850506
	IHWC cell line		B*							
1	9001 SA		*0702		-	-	-	-	-	-
2	9280 LK707		*5201	*7301	-	-	+	-	-	+
3	9011 E4181324		*52011		-	-	+	-	-	-
4	9275 GU373		*1510	*5301	-	-	-	-	-	-
5	9009 KAS011		*3701		-	-	-	-	-	-
6	9353 SM		*3901	*5101	-	-	+	-	-	-
7	9020 QBL		*1801		-	-	-	-	-	-
8	9007 DEM		*5701		-	-	-	-	-	-
9	9026 YAR		*3801		-	-	-	-	-	-
10	9107 LKT3		*5401		-	+	-	-	+	-
11	9051 PITOUT		*4403		-	-	-	-	-	-
12	9052 DBB		*5701		-	-	-	-	-	-
13	9067 BTB		*2705		-	-	-	-	-	-
14	9071 OLGA		*1501	*1520	-	-	-	-	-	-
15	9075 DKB		*4001		-	-	-	-	-	-
16	9037 SWEIG007		*4002		-	-	-	-	-	-
17	9008 WILJON		*1801		-	-	-	-	-	-
18	9257 32367		*1401	*5601	-	+	-	-	+	-
19	9038 BM16		*1801		-	-	-	-	-	-
20	9059 SLE005		*4001		-	-	-	-	-	-
21	9064 AMALA		*1501		-	-	-	-	-	-
22	9056 KOSE		*3503		-	-	-	-	-	-
23	9124 IHL		*4002	*5602	-	-	-	-	+	-
24	9035 JBUSH		*3801		-	-	-	-	-	-
25	9049 IBW9		*1402		-	-	-	-	-	-
26	9285 WT49		*5801		-	-	-	-	-	-
27	9191 CH1007		*0705	*5101	-	-	+	-	-	-
28	9320 BEL5GB		*4402	*4403	-	-	-	-	-	-
29	9050 MOU		*4403		-	-	-	-	-	-
30	9021 RSH		*4201		-	-	-	-	-	-
31	9019 DUCAF		*1801		-	-	-	-	-	-
32	9297 HAG		*4102		-	-	-	-	-	-
33	9098 MT14B		*4001		-	-	-	-	-	-
34	9104 DHIF		*3801		-	-	-	-	-	-
35	9302 SSTO		*4402		-	-	-	-	-	-
36	9024 KT17		*1501	*3501	-	-	-	-	-	-
37	9065 HHKB		*0702		-	-	-	-	-	-
38	9099 LZL		*1501		-	-	-	-	-	-
39	9315 CML		*0801	*2705	-	-	-	-	-	-
40	9134 WHONP199		*1302	*4601	-	+	-	-	-	-
41	9055 H0301		*1402		-	-	-	-	-	-
42	9066 TAB089		*4601		-	-	-	-	-	-
43	9076 T7526		*4601		-	-	-	-	-	-
44	9057 TEM		*3801		-	-	-	-	-	-
45	9239 SHJO		*4201	*5001	-	+	-	-	-	-
46	9013 SCHU		*0702		-	-	-	-	-	-
47	9045 TUBO		*5101		-	-	+	-	-	-
48	9303 TER-ND		*3501	*4403	-	-	-	-	-	-

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CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-B*59 SSP

Product number: 101.554-06u – without *Taq* polymerase
Lot number: 02F
Expiry date: 2010-August-01
Number of tests: 6
Number of wells per test: 6

Well specifications:

Well No.	Production No.
1	2006-248-01
2	2006-248-02
3	2006-248-03
4	2007-396-04
5	2008-505-06
6	2008-505-06

The specificity of each primer solution of the HLA-B*59 primer set has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solution 4 were available. The specificities of the primers in primer solution 4 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer.

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

Date of approval: 2009-May-27

Approved by:

Quality Control, Supervisor

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Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*59
Product number: 101.554-06u
Lot number: 02F

Intended use: HLA-B*59 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Hasselstigen 1
SE-133 33 Saltsjöbaden, 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 9001:2000 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-27

Olle Olerup
Managing Director

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ADDRESSES:

Manufacturer:

Olerup SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

Tel: +46-8-717 88 27

Fax: +46-8-717 88 18

E-mail: info-ssp@olerup.com

Web page: <http://www.olerup.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**.