

Lot No.: **86F**

Lot-specific Information

***Olerup SSP*[®] HLA-B*45**

Product number: 101.564-06u – without *Taq* polymerase
Lot number: 86F
Expiry date: 2011-May-01
Number of tests: 6
Number of wells per test: 10
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. 86F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] HLA-B*45 LOT.

The HLA-B*45 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP*[®] HLA-B*45 lot was made (Lot No. 16E).

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

Well	5'-primer	3'-primer	rationale
6	-	-	Exchanged positive control primer pair.
9	New	New	New primer pair for the B*4508 allele.
10	New	New	New primer pair for the B*4509 allele.

Lot No.: **86F**

Lot-specific Information

PRODUCT DESCRIPTION

HLA-B*45 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*4501 to B*4509 alleles.

PLATE LAYOUT

Each HLA-B*45 test consists of 10 PCR reactions in an 16 well cut PCR plate. Wells 11 to 16 are empty.

1	2	3	4	5	6	7	8
9	10	empty	empty	empty	empty	empty	empty

The 16 well PCR plate is marked with 'HLA-B*45' in silver/gray ink.

Well No. 1 is marked with the Lot No. '86F'.

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

Please note: When removing each 16 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*45 SSP subtypings will be influenced by four B*08, most B*13, thirteen B*15, the B*2714, four B*35, four B*39, most B*40, the B*41, two B*42, seven B*44, two B*46, two B*47, the B*49, the B*50, the B*51, the B*52, the B*54, most B*55, most B*56, the B*5808, the B*59, the B*7301, the B*78 and the B*82 alleles when present on the other haplotype. In addition, the Cw*0768 will be amplified by primer mix 10.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*45, i.e. **B*4501 to B*4509**, recognized by the HLA Nomenclature Committee in May 2009¹ will be amplified by the primers in the HLA-B*45 SSP kit.

¹HLA-B alleles listed on the IMGT/HLA web page 2009-May-11, release 2.25.2, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 9 HLA-B*45 alleles can be combined in 45 homozygous and heterozygous combinations. Eighteen of these genotypes do not give rise to unique amplification patterns.

+++++--- +-4503, 4508 = 4507, 4508
+++++--- --4503, 4504 = 4504, 4507
+++++--- --4503, 4505 = 4505, 4507
+++++--- --4503, 4506 = 4506, 4507
+++++--- --4501, 4503 = 4501, 4507 = 4503, 4507 = 4507, 4507
+++++--- --4501, 4505 = 4505, 4505
+++++--- --4501, 4506 = 4506, 4506
-+++++--- +-4504, 4508 = 4508, 4508

Lot No.: **86F**

Lot-specific Information

SPECIFICITY TABLE

HLA-B*45 SSP subtyping

Specificities and sizes of the PCR products of the 10 primer mixes used for HLA-B*45 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*45 alleles	Other amplified HLA-B alleles ³
1	255 bp	800 bp	4501-4503, 4505-4507, 4509	4415, 4418, 4420, 4447, 5002, 5123
2	145 bp	1070 bp	4501-4509	1546, 1553, 9506, 9543, 3519, 3547, 3563, 400101-4011, 401401-4016, 4018, 4020, 4022N-4040, 4042-4045, 4048-4075, 4077-4092, 4094, 4095, 4101-4104, 4106-4108, 4409, 4446, 4702, 4703 ^{weakly} , 5001, 5002, 5004
3	160 bp	800 bp	4501, 4504-4508	4415, 4418, 5613, 8201, 8202
4	160 bp	800 bp	4503, 4507	
5	195 bp	800 bp	4502	
6	255 bp	1070 bp	4504, 4508	0809, 130201-1304, 1308Q, 1309, 1314-1316, 1318, 1319, 1504, 1516, 1542, 1567, 1583, 1595, 9537, 9555, 2714, 3537, 3560, 390601-390602, 3933, 3934, 40060101-400602, 4044, 4053, 4070, 4075, 4083, 4086, 4093, 4095, 4096, 4101, 4105-4107, 4204, 4611, 4618, 490101-4905, 5001, 5004, 510101-5103, 5105, 5107-5122, 512401-512402,

Lot No.: **86F**

Lot-specific Information

				5126-5141N, 5143, 5144N, 5148-5155, 5157, 5158, 5160, 5161, 5163, 5165, 520101-5214, 5401-5405N, 5407, 5408N, 5410-5417, 550101-5503, 5505, 5507, 5509-5513, 5515-5534, 5601, 560501-5608, 5613-5617, 5619N-5624, 5808, 5901-5904, 7301, 7801-7806
7	230 bp	1070 bp	4505	
8	105 bp	1070 bp	4506	
9	245 bp	1070 bp	4508	0809, 1542, 1583, 3560, 390601-390602, 3934, 4101, 4105-4107, 4204, 4618, 5121, 5136, 5401, 5402, 5404, 5405N, 5407, 5408N, 5410-5417, 550101-5503, 5505, 5507, 5510-5513, 5515-5521, 5523, 5525-5534, 5623, 5901-5903
10	160 bp	1070 bp	4509	0828, 0835, 0837, 1551, 4209, 4417, Cw*0768

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*45 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.

Lot No.: **86F**

Lot-specific Information

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 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*45 SSP subtyping.

In addition, wells number 3, 4 and 5 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

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 several non-HLA-B*45 alleles will be amplified by primer mixes 1 to 3, 6, 9 and 10.

Lot No.: **86F**

Lot-specific Information

Interpretation Table										
HLA-B*45 SSP subtyping										
Amplification patterns of the B*4501 to 4509 alleles.										
	Well									
	1	2	3	4	5	6	7	8	9	10
Length of spec.	255	145	160	160	195	255	230	105	245	160
PCR product										
Length of int.	800	1070	800	800	800	1070	1070	1070	1070	1070
pos. control¹										
5'-primer(s)²	357	206	420	416	420	357	357	206	357	419
	5'-Tgg3'	5'-gAA3'	5'-TTA3'	5'-CCg3'	5'-TTC3'	5'-Tgg3'	5'-Tgg3'	5'-gAA3'	5'-Tgg3'	5'-gTC3'
				662						
				5'-CCg3'						
3'-primer(s)³	572	311	538	538	572	572	545	272	559	538
	5'-gCg3'	5'-ggT3'	5'-gTC3'	5'-gTC3'	5'-gCg3'	5'-gCC3'	5'-AgA3'	5'-TgT3'	5'-CgT3'	5'-gT C 3'
				774						
				5'-ggT3'						
Well No.	1	2	3	4	5	6	7	8	9	10
HLA-B allele										
*4501	1	2	3							
*4502	1	2			5					
*4503	1	2		4						
*4504		2	3			6				
*4505	1	2	3				7			
*4506	1	2	3					8		
*4507	1	2	3	4						
*4508		2	3			6			9	
*4509	1	2								10
*0809, 1542, 1583, 3560, 390601-390602, 3934, 4105, 4204, 4618, 5121, 5136, 5401, 5402, 5404, 5405N, 5407, 5408N, 5410-5417, 550101-5503, 5505, 5507, 5510-5513, 5515-5521, 5523, 5525-5534, 5623, 5901-5903						6			9	
*0828, 0835, 0837, 1551, 4209, 4417										10
Well No.	1	2	3	4	5	6	7	8	9	10

Lot No.: **86F**

Lot-specific Information

Length of spec.	255	145	160	160	195	255	230	105	245	160
PCR product										
Well No.	1	2	3	4	5	6	7	8	9	10
*130201-1304, 1308Q, 1309, 1314-1316, 1318, 1319, 1504, 1516, 1567, 1595, 9537, 9555, 2714, 3537, 3933, 4093, 4096, 4611, 490101-4905, 510101-5103, 5105, 5107-5120, 5122, 512401-512402, 5126-5135, 5137-5141N, 5143, 5144N, 5148-5155, 5157, 5158, 5160, 5161, 5163, 5165, 520101-5214, 5403, 5509, 5522, 5524, 5601, 560501-5608, 5614-5617, 5619N-5622, 5624, 5808, 5904, 7301, 7801-7806						6				
*1546, 1553, 9506, 9543, 3519, 3547, 3563, 400101-4005, 4007-4011, 401401-4016, 4018, 4020, 4022N-4040, 4042, 4043, 4045, 4048-4052, 4054-4069, 4071-4074, 4077-4082, 4084, 4085, 4087-4092, 4094, 4102-4104, 4108, 4409, 4446, 4702		2								
*40060101-400602, 4044, 4053, 4070, 4075, 4083, 4086, 4095, 5001, 5004		2				6				
*4101, 4106, 4107		2				6			9	
*4415, 4418	1		3							
*4420, 4447, 5123	1									
*4703		w								
*5002	1	2								
*5613			3			6				
*8201, 8202			3							
HLA-B allele										
Cw*0768										10
Well No.	1	2	3	4	5	6	7	8	9	10

Lot No.: **86F**

Lot-specific Information

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 in order to help in the correct orientation of the HLA-B*45 SSP subtyping.

In addition, wells number 3, 4 and 5 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

²The nucleotide position, in the 1st, 2nd 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.

'w', may be weakly amplified.

Lot No.: **86F**

Lot-specific Information

CELL LINE VALIDATION SHEET															
HLA-B*45 SSP primer set															
					Well										
					1	2	3	4	5	6	7	8	9	10	
					Prod. No.:	200621301	200621302	200959603	200621304	200621305	200959606	200621307	200621308	200959609	200959610
IHC 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	9025	DEU	*3501		-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*3801		-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*5401		-	-	-	-	-	+	-	-	+	-	-
11	9051	PITOUT	*4403		-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*5701		-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*2705		-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*1501	*1520	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*4001		-	+	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*4002		-	+	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*0801	*5501	-	-	-	-	-	+	-	-	+	-	-
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	-	-	-	-	-	-	-	-	-	-	-

Lot No.: **86F**

Lot-specific Information

CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-B*45 SSP

Product number: 101.564-06u – without *Taq* polymerase
Lot number: 86F
Expiry date: 2011-May-01
Number of tests: 6
Number of wells per test: 10

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2006-213-01	9	2009-596-09
2	2006-213-02	10	2009-596-10
3	2009-596-03		
4	2006-213-04		
5	2006-213-05		
6	2009-596-06		
7	2006-213-07		
8	2006-213-08		

The specificity of each primer solution of the HLA-B*45 primer set has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 4, 5, 7, 8 and 10 were available. The specificities of the primers in primer solutions 5, 8 and 10 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer mix 4 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer mix 7 it was only possible to test the 5'-primer, the 3'-primer was not possible to test.

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

Date of approval: 2009-May-27

Approved by:

Quality Control, Supervisor

Lot No.: **86F**

Lot-specific Information

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*45
Product number: 101.564-06u
Lot number: 86F

Intended use: HLA-B*45 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

Lot No.: **86F**

Lot-specific Information

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**.