Olerup SSP® HLA-B*5701

Product number:	**101.572-12 – including *Taq* polymerase**
Lot number:	**88F**
Expiry date:	**2011-April-01**
Number of tests:	**12**
Number of wells per test:	**8**
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. 88F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® HLA-B*5701 LOT.

The HLA-B*5701 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup* SSP® HLA-B*5701 lot was made **(Lot No. 83E)**.

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

Well	5'-primer	3'-primer	rationale
3	-	Added	New 3'-primer for the B*5718 allele

PRODUCT DESCRIPTION

HLA-B*5701 SSP subtyping

CONTENT
The primer set contains 5'- and 3'-primers for identifying the B*570101 to 570103 alleles.

PLATE LAYOUT
Each test consists of 8 PCR reactions in an 8 well cut PCR plate.

1	2	3	4	5	6	7	8

The 8 well cut PCR plate is marked with '5701' in silver/gray ink.
Well No. 1 is marked with the Lot No. '88F'.
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*5701 SSP subtypings will influence by the other B*57 alleles and also by the B*4030, 4034, 5515 and 5814 alleles.

UNIQUELY IDENTIFIED ALLELES
HLA-B*5701 will give rise to a unique amplification pattern by the primers in the HLA-B*5701 kit[1].
The HLA-B*5701 typing kit cannot distinguish the B*570101 to B*570103 alleles.

[1]HLA-B alleles listed on the IMGT/HLA web page 2009-January-16, release 2.24.0, www.ebi.ac.uk/imgt/hla.

SPECIFICITY TABLE

HLA-B*5701 SSP subtyping

Specificities and sizes of the PCR products of the 8 primer mixes used for HLA-B*5701 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified HLA-B*57 alleles	Other amplified HLA-B alleles[3]
1[4]	90 bp	**800 bp**	570101-5715, 5717-5719	
2	220 bp	**800 bp**	570101-570103, 570301-570302, 5706-5708, 5710, 5714-5718	4030, 4034, 5514, 5814
3[4,6]	95, 170 bp	**800 bp**	5704, 5706, 5718	
4[4,5]	105 bp	1070 bp	5715	
5[4,7]	90, 250 bp	**800 bp**	5707, 5716	
6[4,8]	90, 210 bp	1070 bp	5702-570302, 5707-5709, 5712, 5717, 5717	4030, 4034
7[9]	165, 215 bp	1070 bp	5709, 5714	5514, 5814
8	195 bp	1070 bp	5710	

[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 HLA-B*5701 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 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*5701 subtyping.

In addition, wells number 2, 3 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.
[3]Due to the sharing of sequence motifs between HLA-B alleles some non-HLA-B*57 alleles will be amplified by primer mixes 2, 6 and 7.
[4]Short specific PCR fragments are less intense and not as sharp as longer specific bands.
[5]Primer mix 4 has a tendency of giving rise to nonspecific amplifications.
[6]Primer mix 3: Specific PCR fragment of 95 bp in the B*5704 allele. Specific PCR fragment of 170 bp in the B*5706 and B*5718 allele.
[7]Primer mix 5: Specific PCR fragment of 90 bp in the B*5716 allele. Specific PCR fragment of 250 bp in the B*5707 allele.
[8]Primer mix 6: Specific PCR fragment of 90 bp in the B*5702-570302, 5707, 5709, 5712, 5717 and the B*4030 and 4034 alleles. Specific PCR fragment of 210 bp in the B*5708 allele.
[9]Primer mix 7: Specific PCR fragment of 165 bp in the B*5714 and B*5514 and 5814 alleles. Specific PCR fragment of 215 bp in the B*5709 allele.

INTERPRETATION TABLE

HLA-B*5701 SSP typing

	Well[4]							
	1	2	3	4	5	6	7	8
Length of spec.	90	220	95	105	90	90	165	195
PCR product			170		250	210	215	
Length of int.	800	800	800	1070	800	1070	1070	1070
pos. control[1]								
5'-primer(s)[2]	209	362	362	209	209	320	362	103
	5'-ggC3'	5'-ggT3'	5'-ggT3'	5'-ggC3'	5'-ggA3'	5'-CCC3'	5'-ggT3'	5'-CCT3'
					362	362		
					5'-ggT3'	5'-ggT3'		
3'-primer(s)[3]	256	539	418	271	256	2nd I	486	256
	5'-CCC3'	5'-TCA3'	5'-gTC3'	5'-CAC3'	5'-CCC3'	5'-TCg3'	5'-gCg3'	5'-CCC3'
			500		572	412	538	
			5'-ggA3'		5'-gCg3'	5'-gTT3'	5'-gTC3'	
Well No.	1	2	3	4	5	6	7	8
HLA-B allele								
*570101-570103	1	2						
*5702, 5712	1					6		
*570301-570302, 5708, 5717	1	2				6		
*5704	1		3					
*5705, 5711, 5713, 5719	1							
*5706, 5718	1	2	3					
*5707	1	2			5	6		
*5709	1					6	7	
*5710	1	2						8
*5714	1	2					7	
*5715	1	2		4				
*5716		2			5			
*4030, 4034		2				6		
*5514, 5814		2					7	
HLA-B allele								
Well No.	1	2	3	4	5	6	7	8

[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 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*5701 subtyping.
In addition, wells number 2, 3 and 5 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.
[2]The nucleotide position, in the 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.
[3]The nucleotide position, in the 2nd or 3rd exon or the 2nd intron 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.
[4]Primer mix 3: Specific PCR fragment of 95 bp in the B*5704 allele. Specific PCR fragment of 180 bp in the B*5706 and B*5718 alleles.
Primer mix 5: Specific PCR fragment of 90 bp in the B*5716 allele. Specific PCR fragment of 250 bp in the B*5707 allele.
Primer mix 6: Specific PCR fragment of 90 bp in the B*5702-570302, 5707, 5709, 5712, 5717 and the B*4030 and 4034 alleles. Specific PCR fragment of 210 bp in the B*5708 allele.
Primer mix 7: Specific PCR fragment of 165 bp in the B*5714 and B*5514 and 5814 alleles. Specific PCR fragment of 215 bp in the B*5709 allele.

CELL LINE VALIDATION SHEET

HLA-B*5701 SSP typing kit

					Well							
					1	2	3	4	5	6	7	8
				Prod. No.:	200959801	200848702	200959803	200848704	200848705	200959806	200959807	200959808
	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	*440301		-	-	-	-	-	-	-	-
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	*440301		-	-	-	-	-	-	-	-
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	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-B*5701 SSP

Product number: **101.572-12 – including *Taq* polymerase**
Lot number: **88F**
Expiry date: **2011-April-01**
Number of tests: **12**
Number of wells per test: **8**

Well specifications:

Well No.	Production No.
1	2009-598-01
2	2008-487-02
3	2009-598-03
4	2008-487-04
5	2008-487-05
6	2009-598-06
7	2009-598-07
8	2009-598-08

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 3, 4, 5, 7 and 8 were available. The specificities of the primers in primer solutions 3, 5, 7 and 8 were tested by separately adding one or two additional 5'-primer(s), respectively one or two additional 3'-primer(s). In primer solution 4 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solution 3, two 3'-primers were not possible to test, and in primer solution 5, one 5'-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

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*5701
Product number: 101.572-12
Lot number: 88F

Intended use: HLA-B*5701 histocompatibility testing

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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-27

Olle Olerup
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