

Olerup SSP[®] HLA-Cw*08

Product number:	101.623-12 – with <i>Taq</i> polymerase 101.623-12u – without <i>Taq</i> polymerase
Lot number:	23E
Expiry date:	2010-April-01
Number of tests:	12
Number of wells per test:	16
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. 23E.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP[®] HLA-Cw*08 LOT

The HLA-Cw*08 specificity and interpretation tables have been updated for the HLA-Cw alleles described since the previous Olerup SSP[™] HLA-Cw*08 lot was made (**Lot No. X25**).

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	Primer pair added for the Cw*0815 allele.
15	Modified	-	Changed 5'-primer to decrease the tendency to form primer dimer artefacts.
16	Modified	-	Modified primer added for increased yield of specific PCR product.

Changes in revision R01 compared to R00:

1. The Cw*0816 allele is amplified by primer mix 15.

PRODUCT DESCRIPTION

HLA-Cw*08 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the Cw*0801 to Cw*0816 alleles.

PLATE LAYOUT

Each HLA-Cw*08 test consists of 16 PCR reactions in a 16 well cut PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16

The 16 well PCR plate is marked with 'Cw*08'.

Well No. 1 is marked with the Lot No. '23E'.

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-Cw*08 SSP subtypings will be influenced by other HLA-Cw alleles, as primer mixes 1, 3, 5, 8, 9, 11, 13 and 16 amplify non-HLA-Cw*08 alleles. In addition, primer mix 1 will amplify the B*5802 allele and primer mix 5 will amplify the B*1533 allele.

UNIQUELY IDENTIFIED ALLELES

All the HLA-Cw*08 alleles, i.e. **Cw*0801 to Cw*0816**, recognized by the HLA Nomenclature Committee in April 2008¹ will be amplified by the primers in the HLA-Cw*08 SSP kit.

The HLA-Cw*08 subtyping kit cannot distinguish the Cw*080101 to Cw*080102 alleles.

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

RESOLUTION IN HOMO- AND HETEROZYGOTES

The sixteen HLA-Cw*08 alleles can be combined in 136 homozygous and heterozygous combinations. Forty-nine of these genotypes do not give rise to unique amplification patterns.

+++--+-	----++--	0801,0805 = 0805,0816
+++----+	----++--	0801,0807 = 0807,0816
+++-----	----++--	0801,0812 = 0812,0816
+++-----	----++--	0801,0802 = 0802,0816
+++-----	----++--	0801,0804 = 0804,0816
++-+-+--	----++--	0801,0806 = 0803,0806 = 0806,0806 = 0806,0816
++-+-----	----++--	0801,0814 = 0803,0814 = 0814,0814 = 0814,0816
++-+-----	----++--	0801,0803 = 0803,0803 = 0803,0816
++-+-+--	----++--	0801,0815 = 0815,0816
++-----+	-+-+-----	0801,0809 = 0809,0809 = 0809,0811 = 0809,0816
++-----+	----++--	0801,0811 = 0811,0811 = 0811,0816
++-----	+-----	0810,0810 = 0810,0816
++-----	----++--	0801,0808 = 0808,0808 = 0808,0816
++-----	----++--	0801,0801 = 0801,0816
--+-+--+	----++--	0805,0807 = 0807,0815
--+-+--	----++--	0805,0812 = 0812,0815
--+-+--	----++--	0802,0805 = 0802,0815 = 0805,0805 = 0805,0815
--+-----	----++--	0802,0807 = 0807,0807
--+-----	----++--	0802,0812 = 0812,0812

SPECIFICITY TABLE

HLA-Cw*08 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-Cw*08 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-Cw*08 alleles	Other amplified HLA Class I alleles ³
1	250 bp	800 bp	080101-080102, 0803, 0804, 0806, 0808-0811, 0813, 0814, 0816	0203, 0216, 0218, 040401-040402, 0406, 0413, 0511, 0517, 0604, 1214, 1218, 1220, 1406, 150201-1507, 1509-1513, 1515-1521, 1701-1704, B*5802
2⁴	115 bp	1070 bp	080101-080102, 0803, 0806, 0808-0811, 0814, 0816	
3⁴	115 bp	800 bp	0802, 0804, 0805, 0807, 0812, 0813	050101-050104, 0503-0508, 0510, 0511, 0513-0516, 0518, 0519, 0741
4⁴	110 bp	1070 bp	0803, 0806, 0814	
5	155 bp	1070 bp	0805, 0815	0113, 0509, 0517, B*1533
6	270 bp	1070 bp	0806	
7⁴	100 bp	1070 bp	0807	
8	225 bp	1070 bp	0809, 0811	0504
9	165 bp	800 bp	0810	050101-050104, 0503-0519, 0605, 1221
10⁴	110 bp	800 bp	0809	
11	280 bp	800 bp	0812	0516
12	165 bp	800 bp	080101-0809, 0811, 0812, 0814, 0815	
13⁴	105 bp	800 bp	0802, 0805, 0807, 0812	050101-050104, 0503-0507N, 0510, 0512-0516, 0518, 0519, 0741
14	280 bp	1070 bp	0808	

15	260 bp	1070 bp	0813, 0816	
16	100 bp	1070 bp	0814	0605, 1216

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-Cw*08 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 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 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-Cw*08 SSP subtyping.

In addition, wells number 3 and 9 to 13 contain 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-Cw alleles some non-HLA-Cw*08 alleles will be amplified by primer mixes 1, 3, 5, 8, 9, 11, 13 and 16. In addition, primer mix 1 will amplify the B*5802 allele and primer mix 5 will amplify the B*1533 allele.

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

INTERPRETATION TABLE								
HLA-Cw*08 SSP subtyping								
Amplification patterns of the HLA-Cw*0801 to 0816 alleles								
	Well							
	1	2	3	4	5	6	7	8
Length of spec.	250	115	115	110	155	270	100	225
PCR product								
Length of int.	800	1070	800	1070	1070	1070	1070	1070
pos. control¹								
5'-primer²	2nd I	527	527	527	176	2nd I	453	419
	<small>5'-CCA^{3'}</small>	<small>5'-TAC^{3'}</small>	<small>5'-TgA^{3'}</small>	<small>5'-TAC^{3'}</small>	<small>5'-gCA^{3'}</small>	<small>5'-CCA^{3'}</small>	<small>5'-AAT^{3'}</small>	<small>5'-gTC^{3'}</small>
					485			
					<small>5'-CAA^{3'}</small>			
3'-primer³	539	601	601	595	289	559	512	601
	<small>5'-TCA^{3'}</small>	<small>5'-CTT^{3'}</small>	<small>5'-CTT^{3'}</small>	<small>5'-CCT^{3'}</small>	<small>5'-AgC^{3'}</small>	<small>5'-CgC^{3'}</small>	<small>5'-CCA^{3'}</small>	<small>5'-CTT^{3'}</small>
					601			
					<small>5'-CTC^{3'}</small>			
Well No.	1	2	3	4	5	6	7	8
HLA-Cw allele								
*080101-080102	1	2						
*0802			3					
*0803	1	2		4				
*0804	1		3					
*0805			3		5			
*0806	1	2		4		6		
*0807			3				7	
*0808	1	2						
*0809	1	2						8
*0810	1	2						
*0811	1	2						8
*0812			3					
*0813	1		3					
*0814	1	2		4				
*0815					5			
*0816	1	2						
Well No.	1	2	3	4	5	6	7	8

INTERPRETATION TABLE								
HLA-Cw*08 SSP subtyping								
Amplification patterns of the HLA-Cw*0801 to 0816 alleles								
Well								
9	10	11	12	13	14	15	16	
165	110	280	165	105	280	260	100	Length of spec. PCR product
800	800	800	800	800	1070	1070	1070	Length of int. pos. control¹
176	527	361	176	539	363	1st 	142	5'-primer²
5'-gCA ^{3'}	5'-TAC ^{3'}	5'-AgT ^{3'}	5'-gCA ^{3'}	5'-gCg ^{3'}	5'-AgC ^{3'}	5'-CgA ^{3'}	5'-TCT ^{3'}	
302	598	601	302	601	601	175	201	3'-primer³
5'-ggT ^{3'}	5'-CTC ^{3'}	5'-CTT ^{3'}	5'-ggC ^{3'}	5'-CTT ^{3'}	5'-CTT ^{3'}	5'-CCg ^{3'}	5'-CTT ^{3'}	
9	10	11	12	13	14	15	16	Well No.
								HLA-Cw allele
			12					*080101-080102
			12	13				*0802
			12					*0803
			12					*0804
			12	13				*0805
			12					*0806
			12	13				*0807
			12		14			*0808
	10		12					*0809
9								*0810
			12					*0811
		11	12	13				*0812
						15		*0813
			12				16	*0814
			12					*0815
						15		*0816
9	10	11	12	13	14	15	16	Well No.

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Lot-specific information

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Length of spec.	250	115	115	110	155	270	100	225
PCR product								
Well No.	1	2	3	4	5	6	7	8
*0113					5			
*0203, 0216, 0218, 040401-040402, 0406, 0413, 0604, 1214, 1218, 1220, 1406, 150201- 1507, 1509-1513, 1515- 1521, 1701-1704	1							
*050101-050104, 0503, 0505-0507N, 0510, 0513-0515, 0518, 0519					3			
*0504			3					8
*0508					3			
*0509					5			
*0511	1		3					
*0512								
*0516					3			
*0517	1				5			
*0605								
*0741			3					
*1216								
*1221								
HLA-Cw allele								
Well No.	1	2	3	4	5	6	7	8
B*1533					5			
B*5802	1							
Well No.	1	2	3	4	5	6	7	8

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-Cw*08 SSP subtyping. In addition, wells number 3 and 9 to 13 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band.

Lot No.: **23E**

Lot-specific information

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165	110	280	165	105	280	260	100	Length of spec. PCR product
9	10	11	12	13	14	15	16	Well No.
								*0113
								*0203, 0216, 0218, 040401-040402, 0406, 0413, 0604, 1214, 1218, 1220, 1406, 150201- 1507, 1509-1513, 1515- 1521, 1701-1704
9				13				*050101-050104, 0503, 0505-0507N, 0510, 0513-0515, 0518, 0519
9				13				*0504
9								*0508
9								*0509
9								*0511
9				13				*0512
9		11		13				*0516
9								*0517
9							16	*0605
				13				*0741
							16	*1216
9								*1221
								HLA-Cw allele
9	10	11	12	13	14	15	16	Well No.
								B*1533
								B*5802
9	10	11	12	13	14	15	16	Well No.

²The nucleotide position, in the 2nd or 3rd exon or the 1st or 2nd intron, 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. The sequence of the 3 terminal nucleotides of the primer is given.

CELL LINE VALIDATION SHEET																				
HLA-Cw*08 SSP primer set																				
				Prod. No.:	Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
					200629001	200629002	200629003	200629004	200842805	200629006	200629007	200629008	200629009	200629010	200629011	200659012	200629013	200629014	200842815	200842816
	IHWC cell line		Cw*																	
1	9001 SA		*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707		*0701	*1505	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324		*1202		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373		*0304	*0401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011		*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM		*0304	*0702	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL		*0501		-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-
8	9007 DEM		*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR		*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3		*0102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT		*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB		*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9067 BTB		*0102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA		*0102	*0304	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB		*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007		*0202		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9008 WILJON		*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367		*0102	*0705	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16		*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005		*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA		*0303		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE		*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL		*0102	*1502	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH		*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9		*0802		-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-
26	9285 WT49		*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007		*0704	*1505	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB		*0501	*1601	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-
29	9050 MOU		*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH		*1701		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF		*0501		-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-
32	9297 HAG		*1701	*1703	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B		*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF		*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO		*0501		-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-
36	9024 KT17		*0303	*0401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB		*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL		*0303		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML		*0202	*0701	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199		*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301		*0802		-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-
42	9066 TAB089		*0102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526		*0102	*0801	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
44	9057 TEM		*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO		*0602	*1701	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU		*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO		*0704	*1502	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND		*0401	*1601	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-Cw*08 SSP

Product number: 101.623-12 – including *Taq* polymerase
101.623-12u – without *Taq* polymerase
Lot number: 23E
Expiry date: 2010-April-01
Number of tests: 12
Number of wells per test: 16

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2006-290-01	9	2006-290-09
2	2006-290-02	10	2006-290-10
3	2006-290-03	11	2006-290-11
4	2006-290-04	12	2006-290-12
5	2008-428-05	13	2006-290-13
6	2006-290-06	14	2006-290-14
7	2006-290-07	15	2008-428-15
8	2006-290-08	16	2008-428-16

The specificity of each primer solution of the HLA-Cw*08 primer set has been tested against 48 well characterized cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 4, 5, 6, 7, 8, 10, 11, 14, 15 and 16 were available. The specificity of the primers in primer solutions 5, 7, 8, 11, 14, 15 and 16 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 4, 6 and 10 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: 2008-April-29

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® HLA-Cw*08
Product number: 101.623-12, 101.623-12u
Lot number: 23E

Intended use: HLA-Cw*08 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 III, 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.

Saltsjöbaden, Sweden
2008-April-29

Olle Olerup
Managing Director

HLA-Cw*08

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Lot No.: **23E**

Lot-specific information

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ADDRESSES:

Manufacturer:

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