Olerup SSP® HLA-Cw*01

Product number:	**101.621-12 – including *Taq* polymerase** **101.621-12u – without *Taq* polymerase**
Lot number:	**44E**
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. 44E.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® HLA-CW*01 LOT

The HLA-Cw*01 specificity and interpretation tables have been updated for the HLA-Cw alleles described since the previous *Olerup* SSP® HLA-Cw*01 lot was made **(Lot No. X34)**.

Three wells have been added to the HLA-Cw*01 kit, wells **14 to 16**.

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

Well	5'-primer	3'-primer	rationale
2	-	Added	Primer added for the Cw*0115 allele.
3	Added	Added	Primer pair added for the Cw*0114 allele.
4	Added	Added	Primer pair added for the Cw*0116 allele.
5	-	Added	Primer added for the Cw*0120 allele.
7	-	Added	Primer added for the Cw*0120 allele.
8	Modified	-	Increased specificity of specific primer pair.
14	New	New	New primer pair for the Cw*0117 allele.
15	New	New	New primer pair for the Cw*0118 allele.
16	New	New	New primer pair for the Cw*0119 allele.

Changes in revision R01 compared to R00:

1. Fragment size corrected in footnotes to primer mix 13 in the Specificity and Interpretation Tables.

PRODUCT DESCRIPTION

HLA-Cw*01 SSP typing

CONTENT
The primer set contains 5'- and 3'-primers for identifying the Cw*0102 to Cw*0120 alleles.

PLATE LAYOUT
Each **HLA-Cw*01** 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*01'.
Well No. 1 is marked with the Lot No. '44E'.
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
Non HLA-Cw*01 alleles will be amplified by primer mixes 4, 11 and 12. In addition, the B*1533 allele will be amplified by primer mix 12.

UNIQUELY IDENTIFIED ALLELES
All the HLA-Cw*01 alleles, i.e. **Cw*0102 to Cw*0120**, recognized by the HLA Nomenclature Committee in April 2008[1] will be amplified by the primers in the HLA-Cw*01 SSP kit.
The HLA-Cw*01 subtyping kit cannot distinguish the Cw*010201 to Cw*010205 alleles.

[1]HLA-Cw 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 19 HLA-Cw*01 alleles can be combined in 190 homozygous and heterozygous combinations. Ninety of these genotypes do not give rise to unique amplification patterns. In these calculations the different sizes of the PCR products generated by primer mixes 2, 3, 4, 5, 7, and 13 have not been considered.

```
++++----- ----+---     0103,0114 = 0114,0115
++-+---- ----+---     0103,0116 = 0105,0115 = 0115,0116
++--+-+- ----+---     0103,0120 = 0115,0120
++--+--- ----+---     0103,0106 = 0106,0115
++---+-- ----+---     0103,0107 = 0107,0115
++----+- ----+---     0103,0108 = 0108,0115
++-----+ ----+---     0103,0109 = 0109,0115
++------ +---+---     0103,0110 = 0110,0115
++------ -+--+---     0103,0111 = 0111,0115
```

```
++------ ---++---     0103,0113 = 0113,0115
++------ ----++--     0103,0117 = 0115,0117
++------ ----+-+-     0103,0118 = 0115,0118
++------ ----+--+     0103,0119 = 0115,0119
++------ ----+---     0102,0103 = 0102,0115 = 0103,0115 =
                     0115,0115
+-++---- ----+---     0105,0114 = 0114,0116
+-+----+ ----+---     0102,0104 = 0104,0109 = 0104,0114 =
                     0109,0114
+-+----- ----+---     0102,0114 = 0114,0114
+--++-+- ----+---     0105,0120 = 0116,0120
+--++--- ----+---     0105,0106 = 0106,0116
+--+-+-- ----+---     0105,0107 = 0107,0116
+--+--+- ----+---     0105,0108 = 0108,0116
+--+---+ ----+---     0105,0109 = 0109,0116
+--+---- +---+---     0105,0110 = 0110,0116
+--+---- -+--+---     0105,0111 = 0111,0116
+--+---- ---++---     0105,0113 = 0113,0116
+--+---- ----++--     0105,0117 = 0116,0117
+--+---- ----+-+-     0105,0118 = 0116,0118
+--+---- ----+--+     0105,0119 = 0116,0119
+--+---- ----+---     0102,0105 = 0102,0116 = 0105,0116 =
                     0116,0116
+---+-+- ----+---     0102,0120 = 0106,0108 = 0106,0120 =
                     0108,0120 = 0120,0120
+---+--- ----+---     0102,0106 = 0106,0106
+----+-- ----+---     0102,0107 = 0107,0107
+-----+- ----+---     0102,0108 = 0108,0108
+------+ ----+---     0102,0109 = 0109,0109
+------- +---+---     0102,0110 = 0110,0110
+------- -+--+---     0102,0111 = 0111,0111
+------- ---++---     0102,0113 = 0113,0113
+------- ----++--     0102,0117 = 0117,0117
+------- ----+-+-     0102,0118 = 0118,0118
+------- ----+--+     0102,0119 = 0119,0119
```

SPECIFICITY TABLE

HLA-Cw*01 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-Cw*01 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified HLA-Cw*01 alleles	Other amplified HLA Class I alleles[3]
1[4]	90 bp	**800 bp**	010201-010205, 0106-0111, 0113-0120	
2[4,5]	90, 270 bp	1070 bp	0103, 0115	
3[6]	150, 265 bp	**800 bp**	0104, 0114	
4[7]	210, 240 bp	1070 bp	0105, 0116	0737
5[8]	150, 200 bp	**800 bp**	0106, 0120	
6	195 bp	1070 bp	0107	
7[9]	150, 195 bp	1070 bp	0108, 0120	
8	210 bp	1070 bp	0104, 0109	
9	230 bp	**800 bp**	0110	
10	290 bp	1070 bp	0111	
11	340 bp	**800 bp**	0112	0617, 120306, 1611
12	155 bp	1070 bp	0113	0509, 0517, 0815, **B*1533**
13[10]	155, 370 bp	**800 bp**	010201-0120	
14	240 bp	1070 bp	0117	
15	230 bp	**800 bp**	0118	
16	255 bp	1070 bp	0119	

[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-Cw*01 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 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-Cw*01 SSP subtyping.
In addition, wells number 3, 5, 9, 11, 13 and 15 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 Class I alleles some non-HLA-Cw*01 alleles will be amplified by primer mixes 4, 11 and 12. In addition, the B*1533 allele will be amplified by primer mix 12.
[4]Short specific PCR fragments are less intense and not as sharp as longer specific bands.
[5]Primer mix 2: Specific PCR product of 90 bp in the Cw*0103 allele. Specific PCR product of 270 bp in the Cw*0115 allele.
[6]Primer mix 3: Specific PCR product of 150 bp in the Cw*0104 allele. Specific PCR product of 265 bp in the Cw*0114 allele.
[7]Primer mix 4: Specific PCR product of 210 bp in the Cw*0105 and *0737 alleles. Specific PCR product of 240 bp in the Cw*0116 allele.
[8]Primer mix 5: Specific PCR product of 150 bp in the Cw*0120 allele. Specific PCR product of 200 bp in the Cw*0106 allele.
[9]Primer mix 7: Specific PCR product of 150 bp in the Cw*0120 allele. Specific PCR product of 195 bp in the Cw*0108 allele.
[10]Primer mix 13: Specific PCR fragment of 155 bp in the Cw*0105 and *0112 alleles. Specific PCR fragments of 155 and 370 bp in the Cw*010201 to *0104, *0106 to *0111 and *0113 to *0120 alleles (both specific PCR fragments may not always be obtained).

INTERPRETATION TABLE

HLA-Cw*01 SSP subtyping

Amplification patterns of the Cw*0101 to Cw*0120 alleles

	Well[5]							
	1	2	3	4	5	6	7	8
Length of spec.	90	90	150	210	150	195	150	210
PCR product(s)		270	265	240	200		195	
Length of int.	800	1070	800	1070	800	1070	1070	1070
pos. control[1]								
5'-primer(s)[2]	368	368	89	89	368	406	368	368
	5'-gTg3'	5'-gTg3'	5'-gAA3'	5'-gAA3'	5'-gTg3'	5'-gCA3'	5'-gTg3'	5'-gTg3'
			368	368				
			5'-gTg3'	5'-gTg3'				
3'-primer(s)[3]	419	419	312	287	479	559	479	538
	5'-CgT3'	5'-CgA3'	5'-AgT3'	5'-TCg3'	5'-CCA3'	5'-CgT3'	5'-CCA3'	5'-CCA3'
		595	477	538	527		523	
		5'-Cct3'	5'-gCA3'	5'-CCg3'	5'-CCA3'		5'-ACA3'	
Well No.	1	2	3	4	5	6	7	8
HLA-Cw allele[4]								
*010201-010205	1							
*0103		2						
*0104			3					8
*0105				4				
*0106	1				5			
*0107	1					6		
*0108	1						7	
*0109	1							8
*0110	1							
*0111	1							
*0112								
*0113	1							
*0114	1		3					
*0115	1	2						
*0116	1			4				
*0117	1							
*0118	1							
*0119	1							
*0120	1				5		7	
*0509, 0517, 0815								
*0617, 120306, 1611								
*0737				4				
HLA-Cw allele[4]								
B*1533								
Well No.	1	2	3	4	5	6	7	8

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INTERPRETATION TABLE

HLA-Cw*01 SSP subtyping

Amplification patterns of the Cw*0101 to Cw*0120 alleles

Well[5]								
9	10	11	12	13	14	15	16	
230	290	340	155	155	240	230	255	Length of spec.
				370				PCR product(s)
800	1070	800	1070	800	1070	800	1070	Length of int.
								pos. control[1]
368	368	368	485	89	89	89	89	5'-primer(s)[2]
5'-gTg3'	5'-gTg3'	5'-gTA3'	5'-CAA3'	5'-gAA3'	5'-gAA3'	5'-gAA3'	5'-gAA3'	
				368				
				5'-gTg3'				
559	619	3rd I	601	201	289	278	301	3'-primer(s)[3]
5'-CTC3'	5'-ACT3'	5'-ATg3'	5'-CTC3'	5'-CTC3'	5'-AgC3'	5'-ggT3'	5'-gCA3'	
				3rd I				
				5'-CTC3'				
9	10	11	12	13	14	15	16	Well No.
								HLA-Cw allele[4]
				13				*010201-010205
				13				*0103
				13				*0104
				13				*0105
				13				*0106
				13				*0107
				13				*0108
				13				*0109
9				13				*0110
	10			13				*0111
		11		13				*0112
			12	13				*0113
				13				*0114
				13				*0115
				13				*0116
				13	14			*0117
				13		15		*0118
				13			16	*0119
				13				*0120
			12					*0509, 0517, 0815
		11						*0617, 120306, 1611
								*0737
								HLA-Cw allele[4]
			12					B*1533
9	10	11	12	13	14	15	16	Well No.

CE

[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-Cw*01 SSP subtyping.
In addition, wells number 3, 5, 9, 11, 13 and 15 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 3rd 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]The HLA-Cw*0101 nucleotide sequence has been shown to be identical to Cw*0102.
[5]Primer mix 2: Specific PCR product of 90 bp in the Cw*0103 allele. Specific PCR product of 270 bp in the Cw*0115 allele.
Primer mix 3: Specific PCR product of 150 bp in the Cw*0104 allele. Specific PCR product of 265 bp in the Cw*0114 allele.
Primer mix 4: Specific PCR product of 210 bp in the Cw*0105 and *0737 alleles. Specific PCR product of 240 bp in the Cw*0116 allele.
Primer mix 5: Specific PCR product of 150 bp in the Cw*0120 allele. Specific PCR product of 200 bp in the Cw*0106 allele.
Primer mix 7: Specific PCR product of 150 bp in the Cw*0120 allele. Specific PCR product of 195 bp in the Cw*0108 allele.
Primer mix 13: Specific PCR fragment of 155 bp in the Cw*0105 and *0112 alleles. Specific PCR fragments of 155 and 370 bp in the Cw*010201 to *0104, *0106 to *0111 and *0113 to *0120 alleles (both specific PCR fragments may not always be obtained).

CELL LINE VALIDATION SHEET

HLA-Cw*01 SSP primer set

					Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.:	200617401	200844802	200844803	200844804	200844805	200617406	200844807	200844808	200844809	200617410	200844811	200617412	200729813	200844814	200844815	200844816
	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	*0102	*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*01 SSP

Product number: **101.621-12 – including *Taq* polymerase**
101.621-12u – without *Taq* polymerase
Lot number: **44E**
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	2006-174-01	9	2008-448-09
2	2008-448-02	10	2006-174-10
3	2008-448-03	11	2008-448-11
4	2008-448-04	12	2006-174-12
5	2008-448-05	13	2007-298-13
6	2006-174-06	14	2008-448-14
7	2008-448-07	15	2008-448-15
8	2008-448-08	16	2008-448-16

The specificity of each primer solution of the HLA-Cw*01 primer set has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 2 to 12 and 14 to 16 were available. The specificity of the primers in primer solutions 2, 3, 4, 5, 8, 9, 11, 12 and 14 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 6 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solutions 7, 10, 15 and 16 it was only possible to test the 5'-primers, the 3'-primers were not possible to test.

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

Date of approval: 2008-May-16

Approved by:

Quality Control, Supervisor

CE

Declaration of Conformity

Product name: *Olerup* SSP® HLA-Cw*01
Product number: 101.621-12, 101.621-12u
Lot number: 44E

Intended use: HLA-Cw*01 high 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, ISO 17025:1999 and ISO 13485:2000, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex III.

The Technical Construction File is maintained at *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

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