

Olerup SSP[®] HLA-Cw*01

Product number:	101.621-12u – without <i>Taq</i> polymerase
Lot number:	03H
Expiry date:	2012-March-01
Number of tests:	12
Number of wells per test:	24
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. 03H.

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. 68F).

Eight wells has been added to the HLA-Cw*01 kit,
wells **17** to **24**.

The amplification patterns for some rare HLA-Cw*01 alleles
only differ by the length of the specific PCR products.

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	Modified	Modified	Modified primer pair for improved specificity.
8	Modified	-	Modified 5'-primer for improved specificity.
10	Modified	-	Modified 5'-primer for improved specificity.
16	-	Added	Primer added for the Cw*0123 allele.
17	New	New	New primer pair for the Cw*0124 and Cw*0125 alleles.
18	New	New	New primer pair for the Cw*0126 and Cw*0134 alleles.
19	New	New	New primer pair for the Cw*0127 allele.

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20	New	New	New primer pair for the Cw*0128 allele.
21	New	New	New primer pair for the Cw*0129 and Cw*0133 alleles.
22	New	New	New primer pair for the Cw*0130 allele.
23	New	New	New primer pair for the Cw*0131 allele.
24	New	New	New primer pair for the Cw*0132 allele.

PRODUCT DESCRIPTION

HLA-Cw*01 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the Cw*0102 to Cw*0134 alleles.

PLATE LAYOUT

Each **HLA-Cw*01** test consists of 24 PCR reactions in a 24 well cut PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24

The 24 well PCR plate is marked with ‘HLA-Cw*01’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘03H’.

A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.

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

Please note: When removing each 24 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 2, 4, 8, 12 and 20. In addition, the B*0777 allele will be amplified by primer mix 4, the B*1533 allele will be amplified by primer mix 12, the B*5418 allele will be amplified by primer mix 13 and the A*0124 allele will be amplified by primer mix 16.

UNIQUELY IDENTIFIED ALLELES

All the HLA-Cw*01 alleles, i.e. **Cw*0102 to Cw*0134**, recognized by the HLA Nomenclature Committee in January 2010¹ 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*010209 alleles.

The Cw*0129 and Cw*0133 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 21.

¹HLA-Cw alleles listed on the IMGT/HLA web page 2010-January-15, release 2.28.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 33 HLA-Cw*01 alleles give rise to 32 different patterns, as the Cw*0129 and Cw*0133 alleles have patterns that only differ in size of specific PCR product. These can be combined in 528 homozygous and heterozygous combinations. 213 of these genotypes do not give rise to unique amplification patterns. The different sizes of the PCR products generated by primer mixes 2 to 5, 7, 10, 16, 18 and 21 have not been considered in these calculations.

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+++-----	-----+---	-----	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
++-+-----	-----+---	-----	0103,0122 = 0115,0122
++-+-----	-----+---	-----	0103,0109 = 0109,0115
++-+-----	-----+---	-----	0103,0110 = 0110,0115
++-+-----	-----+---	-----	0103,0111 = 0111,0115
++-+-----	-----+---	-----	0103,0113 = 0113,0115
++-+-----	-----+---	-----	0103,0123 = 0115,0123
++-+-----	-----+---	-----	0103,0117 = 0115,0117 = 0115,0121
++-+-----	-----+---	-----	0103,0118 = 0115,0118
++-+-----	-----+---	-----	0103,0119 = 0115,0119
++-+-----	-----+---	-----	0102,0124 = 0103,0125 = 0115,0124 =
			0115,0125 = 0124,0125
++-+-----	-----+---	-----	0103,0126 = 0115,0126
++-+-----	-----+---	-----	0103,0127 = 0115,0127
++-+-----	-----+---	-----	0103,0128 = 0115,0128
++-+-----	-----+---	-----	0103,0129 = 0115,0129
++-+-----	-----+---	-----	0103,0130 = 0115,0130
++-+-----	-----+---	-----	0103,0131 = 0115,0131
++-+-----	-----+---	-----	0103,0132 = 0115,0132
++-+-----	-----+---	-----	0102,0103 = 0102,0115 = 0103,0115 =
			0115,0115
+-+-----	-----+---	-----	0105,0114 = 0114,0116
+-+-----	-----+---	-----	0104,0111 = 0104,0122 = 0114,0122
+-+-----	-----+---	-----	0102,0104 = 0104,0109 = 0104,0114 =
			0109,0114
+-+-----	-----+---	-----	0114,0117 = 0114,0121
+-+-----	-----+---	-----	0102,0114 = 0114,0114
+-+-----	-----+---	-----	0105,0120 = 0116,0120
+-+-----	-----+---	-----	0105,0106 = 0106,0116
+-+-----	-----+---	-----	0105,0107 = 0107,0116
+-+-----	-----+---	-----	0105,0108 = 0108,0116
+-+-----	-----+---	-----	0105,0122 = 0116,0122
+-+-----	-----+---	-----	0105,0109 = 0109,0116
+-+-----	-----+---	-----	0105,0110 = 0110,0116
+-+-----	-----+---	-----	0105,0111 = 0111,0116
+-+-----	-----+---	-----	0105,0113 = 0113,0116
+-+-----	-----+---	-----	0105,0123 = 0116,0123
+-+-----	-----+---	-----	0105,0117 = 0116,0117 = 0116,0121
+-+-----	-----+---	-----	0105,0118 = 0116,0118
+-+-----	-----+---	-----	0105,0119 = 0116,0119
+-+-----	-----+---	-----	0105,0125 = 0116,0125
+-+-----	-----+---	-----	0105,0126 = 0116,0126
+-+-----	-----+---	-----	0105,0127 = 0116,0127
+-+-----	-----+---	-----	0105,0128 = 0116,0128
+-+-----	-----+---	-----	0105,0129 = 0116,0129
+-+-----	-----+---	-----	0105,0130 = 0116,0130
+-+-----	-----+---	-----	0105,0131 = 0116,0131
+-+-----	-----+---	-----	0105,0132 = 0116,0132

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+---+---- -----+--- ------ 0102,0105 = 0102,0116 = 0105,0116 =
0116,0116
+---+--+ -----+--- ------ 0117,0120 = 0120,0121
+---+--+ -----+--- ------ 0102,0120 = 0106,0108 = 0106,0120 =
0108,0120 = 0120,0120
+---+---- -----+--- ------ 0106,0117 = 0106,0121
+---+---- -----+--- ------ 0102,0106 = 0106,0106
+---+--+ -----+--- ------ 0107,0117 = 0107,0121
+---+--+ -----+--- ------ 0102,0107 = 0107,0107
+---+--+ -----+--- ------ 0108,0117 = 0108,0121
+---+--+ -----+--- ------ 0102,0108 = 0108,0108
+---+--+ -+---+--- ------ 0117,0122 = 0121,0122
+---+--+ -+---+--- ------ 0102,0122 = 0109,0111 = 0109,0122 =
0111,0122 = 0122,0122
+---+--+ -----+--- ------ 0109,0117 = 0109,0121
+---+--+ -----+--- ------ 0102,0109 = 0109,0109
+---+--+ +---+--- ------ 0110,0117 = 0110,0121
+---+--+ +---+--- ------ 0102,0110 = 0110,0110
+---+--+ -+---+--- ------ 0111,0117 = 0111,0121
+---+--+ -+---+--- ------ 0102,0111 = 0111,0111
+---+--+ --+---+--- -+----- 0102,0134 = 0112,0126 = 0126,0134
+---+--+ ---+---+--- ------ 0113,0117 = 0113,0121
+---+--+ ---+---+--- ------ 0102,0113 = 0113,0113
+---+--+ ---+---+--- ------ 0117,0118 = 0118,0121
+---+--+ ---+---+--- ------ 0102,0123 = 0117,0119 = 0117,0123 =
0119,0121 = 0119,0123 = 0121,0123 =
0123,0123
+---+--+ -----+--- +----- 0117,0125 = 0121,0125
+---+--+ -----+--- -+----- 0117,0126 = 0121,0126
+---+--+ -----+--- --+----- 0117,0127 = 0121,0127
+---+--+ -----+--- ---+----- 0117,0128 = 0121,0128
+---+--+ -----+--- ----+--- 0117,0129 = 0121,0129
+---+--+ -----+--- ------+--- 0117,0130 = 0121,0130
+---+--+ -----+--- ------+--- 0117,0131 = 0121,0131
+---+--+ -----+--- ------+--- 0117,0132 = 0121,0132
+---+--+ -----+--- ------+--- 0102,0117 = 0102,0121 = 0117,0117 =
0117,0121
+---+--+ -----+--- ------+--- 0102,0118 = 0118,0118
+---+--+ -----+--- ------+--- 0102,0119 = 0119,0119
+---+--+ -----+--- +----- 0102,0125 = 0125,0125
+---+--+ -----+--- -+----- 0102,0126 = 0126,0126
+---+--+ -----+--- --+----- 0102,0127 = 0127,0127
+---+--+ -----+--- ---+----- 0102,0128 = 0128,0128
+---+--+ -----+--- ----+--- 0102,0129 = 0129,0129
+---+--+ -----+--- ------+--- 0102,0130 = 0130,0130
+---+--+ -----+--- ------+--- 0102,0131 = 0131,0131
+---+--+ -----+--- ------+--- 0102,0132 = 0132,0132
-+----- -----+--- +----- 0103,0124 = 0124,0124
----- --+---+--- -+----- 0112,0134 = 0134,0134

0102 = 010201-010209
0129 = 0129 and 0133

SPECIFICITY TABLE

HLA-Cw*01 SSP subtyping

Specificities and sizes of the PCR products of the 24 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 ³	Other amplified HLA Class I alleles ⁴
1⁵	90 bp	800 bp	*010201-010209, 0106-0111, 0113-0120, 0122, 0123, 0125-0133	
2^{5,7}	90 bp, 270 bp	1070 bp	*0103, 0115, 0124	*0358, 0437
3⁸	150 bp, 265 bp	800 bp	*0104, 0114	
4^{6,9}	210 bp, 240 bp	1070 bp	*0105, 0116	*0737, B*0777
5¹⁰	150 bp, 200 bp	800 bp	*0106, 0120	
6	195 bp	1070 bp	*0107	
7^{6,11}	150 bp, 195 bp	1070 bp	*0108, 0120	
8	210 bp	1070 bp	*0104, 0109, 0122	*0623
9	230 bp	800 bp	*0110	
10¹²	210 bp, 290 bp	1070 bp	*0111, 0122	
11	345 bp	800 bp	*0112, 0134	
12	155 bp	1070 bp	*0113	*0509, 0517, 0815, B*1533
13	155 bp	800 bp	*010201-0120, 0122-0134	B*5418
14⁶	240 bp	1070 bp	*0117, 0121, 0123	
15	230 bp	800 bp	*0118	
16¹³	255 bp, 295 bp	1070 bp	*0119, 0123	A*0124
17⁵	75 bp	800 bp	*0124, 0125	
18^{6,14}	195 bp, 260 bp	800 bp	*0126, 0134	
19⁵	100 bp	1070 bp	*0127	
20⁵	110 bp	800 bp	*0128	*0359
21^{5,15}	125 bp, 245 bp	800 bp	*0129, 0133	
22⁶	255 bp	1070 bp	*0130	
23	235 bp	1070 bp	*0131	
24	250 bp	1070 bp	*0132	

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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*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 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 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*01 SSP subtyping.

In addition, wells number 3, 5, 9, 11, 13, 15, 17, 18, 20 and 21 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.

³The Cw*0129 and Cw*0133 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 21.

⁴Due to the sharing of sequence motifs between HLA Class I alleles some non-HLA-Cw*01 alleles will be amplified by primer mixes 2, 4, 8, 12 and 20. In addition, the B*0777 allele will be amplified by primer mix 4, the B*1533 allele will be amplified by primer mix 12, the B*5418 allele will be amplified by primer mix 13 and the A*0124 allele will be amplified by primer mix 16.

⁵Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁶Primer mixes 4, 7, 14, 18 and 22 may have a tendency of giving rise to nonspecific amplifications.

⁷Primer mix 2: Specific PCR product of 90 bp in the Cw*0103 and Cw*0124 and in the Cw*0358 and Cw*0437 alleles. Specific PCR product of 270 bp in the Cw*0115 allele.

⁸Primer mix 3: Specific PCR product of 150 bp in the Cw*0104 allele. Specific PCR product of 265 bp in the Cw*0114 allele.

⁹Primer mix 4: Specific PCR product of 210 bp in the Cw*0105 and Cw*0737 and the B*0777 alleles. Specific PCR product of 240 bp in the Cw*0116 allele.

¹⁰Primer mix 5: Specific PCR product of 150 bp in the Cw*0120 allele. Specific PCR product of 200 bp in the Cw*0106 allele.

¹¹Primer mix 7: Specific PCR product of 150 bp in the Cw*0120 allele. Specific PCR product of 195 bp in the Cw*0108 allele.

¹²Primer mix 10: Specific PCR fragment of 210 bp in the Cw*0122 allele. Specific PCR fragments of 290 bp in the Cw*0111 allele.

¹³Primer mix 16: Specific PCR product of 255 bp in the Cw*0119 allele. Specific PCR product of 295 bp in the Cw*0123 and the A*0124 alleles.

¹⁴Primer mix 18: Specific PCR product of 195 bp in the Cw*0126 allele. Specific PCR product of 260 bp in the Cw*0134 allele.

¹⁵Primer mix 21: Specific PCR fragment of 125 bp in the Cw*0133 allele. Specific PCR fragments of 245 bp in the Cw*0129 allele.

INTERPRETATION TABLE												
HLA-Cw*01 SSP subtyping												
Amplification patterns of the Cw*0101 to Cw*0134 alleles												
	Well⁶											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	90	90	150	210	150	195	150	210	230	210	345	155
PCR product(s)		270	265	240	200		195			290		
Length of int.	800	1070	800	1070	800	1070	1070	1070	800	1070	800	1070
pos. control¹												
5'-primer(s)²	368	368	89	89	368	406	368	368	368	368	368	485
	⁵ -gTg ³	⁵ -gTg ³	⁵ -gAA ³	⁵ -gAA ³	⁵ -gTg ³	⁵ -gCA ³	⁵ -gTg ³	⁵ -gTg ³	⁵ -gTg ³	⁵ -gTg ³	⁵ -gTA ³	⁵ -CAA ³
			368	368								
			⁵ -gTg ³	⁵ -gTC ³								
3'-primer(s)³	419	419	312	287	479	559	479	538	559	539	3 rd I	601
	⁵ -CgT ³	⁵ -CgA ³	⁵ -AgT ³	⁵ -TCg ³	⁵ -CCA ³	⁵ -CgT ³	⁵ -CCA ³	⁵ -CCA ³	⁵ -CTC ³	⁵ -TCA ³	⁵ -ATg ³	⁵ -CTC ³
		595	477	538	527		523	539		619		
		⁵ -CCT ³	⁵ -gCA ³	⁵ -CCg ³	⁵ -CCA ³		⁵ -ACA ³	⁵ -TCA ³		⁵ -TTT ³		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-Cw allele⁴												
*010201-010209	1											
*0103		2										
*0104			3					8				
*0105				4								
*0106	1				5							
*0107	1					6						
*0108	1						7					
*0109	1							8				
*0110	1								9			
*0111	1									10		
*0112											11	
*0113	1											12
*0114	1		3									
*0115	1	2										
*0116	1			4								
*0117	1											
*0118	1											
*0119	1											
*0120	1				5		7					
*0121												
*0122	1							8		10		
*0123	1											
*0124		2										
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE												
HLA-Cw*01 SSP subtyping												
Amplification patterns of the Cw*0101 to Cw*0134 alleles												
Well⁶												
13	14	15	16	17	18	19	20	21	22	23	24	
155	240	230	255	75	195	100	110	125	255	235	250	Length of spec. PCR product(s)
			295		260			245				
800	1070	800	1070	800	800	1070	800	800	1070	1070	1070	Length of int. pos. control¹
89	89	89	89	806	89	368	530	89	368	368	463	5'-primer(s)²
⁵ -gAA ³	⁵ -gAA ³	⁵ -gAA ³	⁵ -gAA ³	⁵ -ggA ³	⁵ -gAA ³	⁵ -gTg ³	⁵ -ggT ³	⁵ -gAA ³	⁵ -gTg ³	⁵ -gTg ³	⁵ -TgA ³	
				818	453			368				
				⁵ -ggC ³	⁵ -AAT ³			⁵ -gTg ³				
201	289	278	301	846	244	427	601	172	583	560	3rd I	3'-primer(s)³
⁵ -CTC ³	⁵ -AgC ³	⁵ -ggT ³	⁵ -gCA ³	⁵ -CAC ³	⁵ -CTg ³	⁵ -gTA ³	⁵ -CTC ³	⁵ -CAT ³	⁵ -gTg ³	⁵ -ACA ³	⁵ -ATg ³	
			341		3rd I			573				
			⁵ -CgT ³		⁵ -ATg ³			⁵ -AgA ³				
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												HLA-Cw allele⁴
13												*010201-010209
13												*0103
13												*0104
13												*0105
13												*0106
13												*0107
13												*0108
13												*0109
13												*0110
13												*0111
13												*0112
13												*0113
13												*0114
13												*0115
13												*0116
13	14											*0117
13		15										*0118
13			16									*0119
13												*0120
	14											*0121
13												*0122
13	14		16									*0123
13				17								*0124
13	14	15	16	17	18	19	20	21	22	23	24	Well No.



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Length of spec.	90	90	150	210	150	195	150	210	230	210	345	155
PCR product(s)		270	265	240	200		195			290		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*0125	1											
*0126	1											
*0127	1											
*0128	1											
*0129, 0133 ⁵	1											
*0130	1											
*0131	1											
*0132	1											
*0134											11	
*0358, 0437		2										
*0359												
*0509, 0517, 0815, B*1533												12
*0623								8				
*0737, B*0777				4								
A*0124												
B*5418												
HLA-Cw allele ⁴												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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*01 SSP subtyping.

In addition, wells number 3, 5, 9, 11, 13, 15, 17, 18, 20 and 21 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 2nd, 3rd or 4th 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, 3rd or 4th 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.

⁴The HLA-Cw*0101 nucleotide sequence has been shown to be identical to Cw*0102.

⁵ The Cw*0129 and Cw*0133 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 21.

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Lot-specific information

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155	240	230	255	75	195	100	110	125	255	235	250	Length of spec. PCR product(s)
			295		260			245				Well No.
13	14	15	16	17	18	19	20	21	22	23	24	
13				17								*0125
13					18							*0126
13						19						*0127
13							20					*0128
13								21				*0129, 0133 ⁵
13									22			*0130
13										23		*0131
13											24	*0132
13					18							*0134
												*0358, 0437
							20					*0359
												*0509, 0517, 0815, B*1533
												*0623
												*0737, B*0777
			16									A*0124
13												B*5418
												HLA-Cw allele ⁴
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

⁶Primer mix 2: Specific PCR product of 90 bp in the Cw*0103 and Cw*0124 and in the Cw*0358 and Cw*0437 alleles. 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 Cw*0737 and the B*0777 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 10: Specific PCR fragment of 210 bp in the Cw*0122 allele. Specific PCR fragments of 290 bp in the Cw*0111 allele.
Primer mix 16: Specific PCR product of 255 bp in the Cw*0119 allele. Specific PCR product of 295 bp in the Cw*0123 and the A*0124 alleles.
Primer mix 18: Specific PCR product of 195 bp in the Cw*0126 allele. Specific PCR product of 260 bp in the Cw*0134 allele.
Primer mix 21: Specific PCR fragment of 125 bp in the Cw*0133 allele. Specific PCR fragments of 245 bp in the Cw*0129 allele.

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.:	201070701	201070702	201070703	201070704	201070705	201070706	201070707	201070708	201070709	201070710	201070711	201070712	201070713	201070714	201070715	201070716
IHCW 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	9025	DEU	*0401		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*0102		+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
11	9051	PITOUT	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*0102		+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
14	9071	OLGA	*0102	*0304	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
15	9075	DKB	*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*0202		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*0303	*0701	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CELL LINE VALIDATION SHEET												
HLA-Cw*01 SSP primer set												
				Well								
				17	18	19	20	21	22	23	24	
				Prod. No.:	201070717	201070718	201070719	201070720	201070721	201070722	201070723	201070724
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	9025	DEU	*0401		-	-	-	-	-	-	-	-
9	9026	YAR	*1203		-	-	-	-	-	-	-	-
10	9107	LKT3	*0102		-	-	-	-	-	-	-	-
11	9051	PITOUT	*1601		-	-	-	-	-	-	-	-
12	9052	DBB	*0602		-	-	-	-	-	-	-	-
13	9004	JESTHOM	*0102		-	-	-	-	-	-	-	-
14	9071	OLGA	*0102	*0304	-	-	-	-	-	-	-	-
15	9075	DKB	*0304		-	-	-	-	-	-	-	-
16	9037	SWEIG007	*0202		-	-	-	-	-	-	-	-
17	9282	CTM3953540	*0303	*0701	-	-	-	-	-	-	-	-
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: 101.621-12u – without *Taq* polymerase
Lot number: 03H
Expiry date: 2012-March-01
Number of tests: 12
Number of wells per test: 24

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2010-707-01	9	2010-707-09	17	2010-707-17
2	2010-707-02	10	2010-707-10	18	2010-707-18
3	2010-707-03	11	2010-707-11	19	2010-707-19
4	2010-707-04	12	2010-707-12	20	2010-707-20
5	2010-707-05	13	2010-707-13	21	2010-707-21
6	2010-707-06	14	2010-707-14	22	2010-707-22
7	2010-707-07	15	2010-707-15	23	2010-707-23
8	2010-707-08	16	2010-707-16	24	2010-707-24

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 24 were available. The specificity of the primers in primer solutions 2 to 5, 8 to 12, 14, 16, 18 and 22 to 24 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 6, 17 and 20 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solutions 7, 15, 19 and 21 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 2, 4, 5, 10, 16 and 18 one of the 3'-primers was not possible to test.

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

Date of approval: 2010-April-06

Approved by:

Quality Control, Supervisor

Lot No.: **03H**

Lot-specific information

www.olerup-ssp.com

Declaration of Conformity

Product name: *Olerup* SSP® HLA-Cw*01
Product number: 101.621-12u
Lot number: 03H

Intended use: HLA-Cw*01 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 9001:2008 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 Construction 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
2010-April-06

Olle Olerup
Managing Director

Lot No.: **03H**

Lot-specific information

www.olerup-ssp.com

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For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.