

Lot No.: **72F**

Lot-specific information

Olerup SSP[®] HLA-Cw*04

Product number: 101.612-12u – without *Taq* polymerase
Lot number: 72F
Expiry date: 2011-April-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. 72F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP[®]* HLA-Cw*04 LOT

The HLA-Cw*04 specificity and interpretation tables have been updated for the HLA-Cw alleles described since the previous *Olerup SSP[®]* HLA-Cw*04 lot was made (**Lot No. 56E**).

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	Added	-	Increased yield of specific PCR product.
8	Added	-	Increased yield of specific PCR product.
9	Added	Added	Primer pair added for the Cw*0435 allele.
12	Moved	Moved, added	Primer pair moved to well 24, primer added for the Cw*0433 allele.
16	Added	-	Primer added for the Cw*0437 allele.
17	Added	-	Increased yield of specific PCR product.
19	Added	Added	Primer pair added for the Cw*0435 and Cw*0437 alleles.
24	Added	Added	Primer pair from well 12.

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PRODUCT DESCRIPTION

HLA-Cw*04 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-Cw*0401 to HLA-Cw*0437 alleles.

PLATE LAYOUT

Each test consists of 24 PCR reactions in a 24 well 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 cut PCR plate is marked with 'HLA-Cw*04' in silver/gray ink.

Well No. 1 is marked with the Lot No. '72F'.

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

The interpretation of HLA-Cw*04 SSP subtypings will be influenced by other HLA-Cw alleles, as primer mixes 1, 4, 5, 7, 8, 11, 14 and 16 amplify non-HLA-Cw*04 alleles. In addition, the B*5802 allele will be amplified by primer mixes 5 and 8.

UNIQUELY IDENTIFIED ALLELES

All the HLA-C*04 alleles, i.e. **Cw*0401 to Cw*0437**, recognized by the HLA Nomenclature Committee in January 2009¹ will give rise to unique amplification patterns by the primers in the HLA-Cw*04 subtyping kit.

The HLA-Cw*04 subtyping kit cannot distinguish the Cw*04010101 to Cw*040106 alleles and the Cw*040401 and Cw*040402 alleles.

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

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 34 HLA-Cw*04 alleles give rise to 35 different amplification patterns that can be combined in 630 homozygous and heterozygous combinations. 412 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR product generated by primer mix 9, 10, 12, 14, 15 and 19 were not considered in these calculations.

+++++---+ ---+----- ----- 0403,0434 = 0406,0408
+++++---+ ---+----- ----- 0406,0429 = 0406,0433
+++++---+ ---+----- ----- 0403,0413 = 0406,0418
+++++---+ ---+----- ----- 0401,0406 = 0403,0404
+++++---+ ---+----- ----- 0403,0429 = 0403,0433

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++++----- --+----- - 0403,0414 = 0403,0428
+++-+--- --+----- - 0407,0434 = 0427,0434 = 0432,0434
+++-+--- --+----- - 0407,0413 = 0413,0427 = 0413,0432
+++-+--- --+----- - 0404,0407 = 0404,0427 = 0404,0432
+++-+--- -++++----- - 0411,0434 = 0434,0436
+++-+--- -++++----- - 0410,0434 = 0431,0434
+++-+--- -++++----- - 0429,0434 = 0433,0434
+++-+--- --+----- - 0408,0413 = 0416,0434 = 0418,0434
+++-+--- --+----- - 0401,0434 = 0404,0408 = 0408,0434
+++-+--- -++++----- - 0411,0413 = 0413,0436
+++-+--- -++++----- - 0404,0411 = 0404,0436
+++-+--- -++++----- - 0410,0413 = 0413,0431
+++-+--- -++++----- - 0404,0410 = 0404,0431
+++-+--- --+----- - 0413,0429 = 0413,0433
+++-+--- --+----- - 0404,0429 = 0404,0433
+++-+--- --+----- - 0401,0413 = 0404,0416 = 0404,0418 =
0413,0416 = 0413,0418
+++----- --+----- - 0405,0407 = 0405,0427 = 0405,0432
+++----- -++++----- - 0405,0411 = 0405,0436
+++----- -++++----- - 0405,0410 = 0405,0431
+++----- -++++----- - 0405,0429 = 0405,0433
+++----- --+----- - 0405,0416 = 0405,0418
+++----- --+----- - 0405,0414 = 0405,0428
+++----- --+----- - 0401,0405 = 0405,0405
+++-----++ --+----- - 0407,0408 = 0408,0427 = 0408,0432
+++-----+ +-+----- --+----- - 0407,0435 = 0427,0435 = 0432,0435
+++-----+ +-+----- -----+ 0407,0430 = 0427,0430 = 0430,0432
+++-----+ +-+----- -----+ 0407,0409N = 0409N,0427 = 0409N,0432
+++-----+ -++++----- - 0407,0411 = 0407,0436 = 0411,0432 =
0427,0436 = 0432,0436
+++-----+ -++++----- - 0407,0410 = 0407,0431 = 0410,0427 =
0410,0432 = 0427,0431 = 0431,0432
+++-----+ --+----- - 0407,0429 = 0407,0433 = 0427,0429 =
0429,0432 = 0432,0433
+++-----+ --+----- - 0407,0412 = 0412,0427 = 0412,0432
+++-----+ --+----- - 0407,0418 = 0416,0432 = 0418,0427 =
0418,0432
+++-----+ --+----- - 0407,0414 = 0407,0428 = 0414,0427 =
0414,0432 = 0427,0428 = 0428,0432
+++-----+ --+-----+ --+----- - 0407,0437 = 0427,0437 = 0432,0437
+++-----+ --+-----+ ----- - 0407,041501 = 041501,0427 =
041501,0432
+++-----+ --+----- +---+----- - 0407,0417 = 0417,0427 = 0417,0432
+++-----+ --+----- -+----- - 0407,0419 = 0419,0427 = 0419,0432
+++-----+ --+----- --+----- - 0407,0420 = 0420,0427 = 0420,0432
+++-----+ --+----- ---+----- - 0407,041502 = 041502,0427 =
041502,0432
+++-----+ --+----- -----+ - 0407,0423 = 0423,0427 = 0423,0432
+++-----+ --+----- -----+ - 0407,0424 = 0424,0427 = 0424,0432
+++-----+ --+----- -----+ - 0407,0425 = 0425,0427 = 0425,0432
+++-----+ --+----- -----+ + 0407,0426 = 0426,0427 = 0426,0432
+++-----+ --+----- ----- - 0401,0407 = 0401,0427 = 0401,0432 =
0407,0432 = 0427,0432 = 0432,0432

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+++-----+ -+++-----	-----	0408,0411 = 0408,0436
+++-----+ -++-----	-----	0408,0410 = 0408,0431
+++-----+ --++-----	-----	0408,0429 = 0408,0433
+++-----+ --+---+-	-----	0408,0416 = 0408,0418
+++-----+ --+---+-	-----	0408,0414 = 0408,0428
+++-----+ --+-----	-----	0401,0408 = 0408,0408

0401 = 040101-040106
0404 = 040401-040402

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SPECIFICITY TABLE

HLA-Cw*04 SSP subtyping

Specificities and sizes of the PCR products of the 24 primer mixes used for HLA-Cw*04 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-Cw*04 alleles	Other amplified HLA Class I alleles ³
1	250 bp	800 bp	04010101- 040106, 0403, 0405, 0407-0412, 041501-0418, 0419 ^{weakly} , 0420, 0423-0433, 0435- 0437	010201-0121, 020201-020203, 020205-020206, 0204-0215, 0217, 0219-0223, 050101-050104, 0503-0510, 0512- 0516, 0518-0523, 06020101- 06020102, 060203, 0603, 0605-0619, 0802, 0805, 0807, 0812, 0815, 0817- 0819, 120201- 1213, 1215-1217, 1221, 140201- 1405, 1407N-1411, 1508, 160401, 1801-1803
2	220 bp	1070 bp	04010101- 040106, 040401- 0405, 0407- 0409N, 0412 ^{weakly} , 0413-041502, 0417-0420, 0423- 0435, 0437	0764
3⁴	145 bp	1070 bp	04010101- 040106, 040401- 0405, 0408- 041502, 0417- 0420, 0423-0426, 0428-0437	
4	210 bp	1070 bp	0403, 0406	0212
5⁵	250 bp	1070 bp	040401-040402, 0406, 0413, 0434	0122, 0203, 0216, 0218, 0511, 0517, 0604, 080101- 080102, 0803,

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				0804, 0806, 0808-0811, 0813, 0814, 0816, 1214, 1218, 1220, 1406, 150201-1507, 1509-1513, 1515-1521, 1701-1705, B*5802
6⁴	95 bp	1070 bp	0405	
7⁴	145 bp	1070 bp	0407, 0427, 0432	0334, 0764, 1803
8⁵	270 bp	1070 bp	0408, 0434	1515, B*5802
9^{4,5,6}	110, 180, 220 bp	1070 bp	0409N, 0430, 0435	
10⁷	195, 220 bp	1070 bp	0410, 0411, 0431, 0436	
11	180 bp	1070 bp	04010101-040106, 0403-0410, 0412-0420, 0423-0426, 0428-0432, 0434-0437	0605
12^{4,8}	125, 180 bp	1070 bp	0411, 0429, 0433, 0436	
13	225 bp	1070 bp	0412	
14⁹	155, 190 bp	1070 bp	0413, 0416, 0418	0708
15^{6,10}	170, 190 bp	1070 bp	0414, 0428	
16⁴	130 bp	1070 bp	041501, 0437	0305, 0325, 0327, 1409
17^{4,6}	90 bp	800 bp	0417	
18	225 bp	1070 bp	0419	
19^{4,11}	120, 155 bp	1070 bp	0420, 0435, 0437	
20	540 bp	1070 bp	041502, 0417	
21⁴	85 bp	1070 bp	0423	
22⁴	130 bp	1070 bp	0424	
23⁴	85 bp	1070 bp	0425	
24	180 bp	1070 bp	0426, 0430	

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*04 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.

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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*04 subtyping.

In addition, well number 17 contains 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-Cw alleles some non-HLA-Cw*04 alleles will be amplified by primer mixes 1, 2, 4, 5, 7, 8, 11, 14 and 16. In addition, HLA-B*5802 will be amplified by primer mixes 5 and 8.

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

⁵The primers in primer mix 5, 8 and 9 may have a tendency of giving rise to nonspecific amplifications. The specific PCR product in primer mix 9 may also be less intense than the other Cw*04 specific PCR products.

⁶Primer mix 9: Specific PCR fragment of 110 bp in the Cw*0435 allele. Specific PCR fragment of 180 bp in the Cw*0430 allele. Specific PCR fragment of 220 bp in the Cw*0409N allele.

⁷Primer mix 10: Specific PCR fragment of 195 bp in the Cw*0431 allele. Specific PCR fragment of 220 bp in the Cw*0410, Cw*0411 and Cw*0436 alleles.

⁸Primer mix 12: Specific PCR fragment of 125 bp in the Cw*0429 and Cw*0436 alleles. Specific PCR fragment of 180 bp in the Cw*0433 allele. Specific PCR fragment of 125 and 180 bp in the Cw*0411 allele.

⁹Primer mix 14: Specific PCR fragment of 155 bp in the Cw*0416 allele. Specific PCR fragment of 190 bp in the Cw*0413, Cw*0418 and Cw*0708 alleles.

¹⁰Primer mix 15: Specific PCR fragment of 170 bp in the Cw*0414 allele. Specific PCR fragment of 190 bp in the Cw*0428 allele.

¹¹Primer mix 19: Specific PCR fragment of 120 bp in the Cw*0435 and Cw*0437 alleles. Specific PCR fragment of 155 bp in the Cw*0420 allele.

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INTERPRETATION TABLE												
HLA-Cw*04 SSP subtyping												
Amplification patterns of the Cw*0401 to Cw*0437 alleles												
	Well ⁵											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	250	220	145	210	250	95	145	270	110	195	180	125
PCR product									180	220		180
									220			
Length of int.	800	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070
pos. control ¹												
5'-primer(s) ²	2 nd	112	112	118	2 nd	98	112	2 nd	391	112	201	218
	5'-CCA ^{3'}	5'-CCT ^{3'}	5'-CCT ^{3'}	5'-CCA ^{3'}	5'-CCA ^{3'}	5'-CTC ^{3'}	5'-CCT ^{3'}	5'-CCA ^{3'}	5'-ACC ^{3'}	5'-CCT ^{3'}	5'-CCA ^{3'}	5'-ggA ^{3'}
							459		1018	368		
							5'-gAT ^{3'}		5'-gTg ^{3'}	5'-gTT ^{3'}		
3'-primer(s) ³	539	289	218	289	539	154	218	559	459	289	341	302
	5'-TCC ^{3'}	5'-AgC ^{3'}	5'-gCT ^{3'}	5'-AgC ^{3'}	5'-TCA ^{3'}	5'-CAg ^{3'}	5'-gCg ^{3'}	5'-CAg ^{3'}	5'-AgA ^{3'}	5'-AgT ^{3'}	5'-CgT ^{3'}	5'-ggC ^{3'}
							559		1052	521		341
							5'-CTC ^{3'}		5'-Tgg ^{3'}	5'-ggA ^{3'}		5'-Cgg ^{3'}
									1092			
									5'-TTA ^{3'}			
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-Cw allele ⁴												
*04010101-040106	1	2	3								11	
*0403	1			4							11	
*040401-040402		2	3		5						11	
*0405	1	2	3			6					11	
*0406				4	5						11	
*0407	1	2					7				11	
*0408	1	2	3					8			11	
*0409N	1	2	3						9		11	
*0410	1		3							10	11	
*0411	1		3							10		12
*0412	1	w	3								11	
*0413		2	3		5						11	
*0414		2	3								11	
*041501	1	2	3								11	
*041502	1	2	3								11	
*0416	1										11	
*0417	1	2	3								11	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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INTERPRETATION TABLE												
HLA-Cw*04 SSP subtyping												
Amplification patterns of the Cw*0401 to Cw*0437 alleles												
Well ⁵												
13	14	15	16	17	18	19	20	21	22	23	24	
225	155	170	130	90	225	120	540	85	130	85	170	Length of spec. PCR product
	190	190				155						
1070	1070	1070	1070	800	1070	1070	1070	1070	1070	1070	1070	Length of int. pos. control ¹
112	105	412	368	2 nd I	368	347	112	368	127	172	89	
5'-CCT ^{3'}	5'-gCT ^{3'}	5'-ATA ^{3'}	5'-gTg ^{3'}	5'-CCA ^{3'}	5'-gTT ^{3'}	5'-gTA ^{3'}	5'-CCT ^{3'}	5'-gTT ^{3'}	5'-ggA ^{3'}	5'-TCC ^{3'}	5'-gAT ^{3'}	
	368	697	369			368					1018	
	5'-gTT ^{3'}	5'-TCC ^{3'}	5'-TAC ^{3'}			5'-gTg ^{3'}					5'-gTg ^{3'}	
						391						
						5'-ACC ^{3'}						
295	218	539	459	379	550	459	368	412	218	218	218	3'-primer(s) ³
5'-TCC ^{3'}	5'-gCT ^{3'}	5'-TCT ^{3'}	5'-AgA ^{3'}	5'-CAC ^{3'}	5'-CAT ^{3'}	5'-AgA ^{3'}	5'-CAT ^{3'}	5'-gTC ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	
	514	846									1052	
	5'-CTT ^{3'}	5'-CAC ^{3'}									5'-Tgg ^{3'}	
	527											
	5'-CCg ^{3'}											
13	14	15	16	17	18	19	20	21	22	23	24	Well No. HLA-Cw allele ⁴
												*04010101-040106
												*0403
												*040401-040402
												*0405
												*0406
												*0407
												*0408
												*0409N
												*0410
												*0411
13												*0412
	14											*0413
		15										*0414
			16									*041501
							20					*041502
	14											*0416
				17			20					*0417
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

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Length of spec.	250	220	145	210	250	95	145	270	110	195	180	125
PCR product									180	220		180
									220			
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*0418	1	2	3								11	
*0419	w	2	3								11	
*0420	1	2	3								11	
*0423	1	2	3								11	
*0424	1	2	3								11	
*0425	1	2	3								11	
*0426	1	2	3								11	
*0427	1	2					7					
*0428	1	2	3								11	
*0429	1	2	3								11	12
*0430	1	2	3						9		11	
*0431	1	2	3							10	11	
*0432	1	2	3				7				11	
*0433	1	2	3									12
*0434		2	3		5			8			11	
*0435	1	2	3						9		11	
*0436	1		3							10	11	12
*0437	1	2	3								11	
*010201-0121, 020201-020203, 020205-020206, 0204-0211, 0213-0215, 0217, 0219-0223, 05010101-050104, 0503-0510, 0512-0516, 0518-0523, 06020101-06020102, 060203, 0603, 0606-0619, 0802, 0805, 0807, 0812, 0815, 0817-0819, 120201-1213, 1215-1217, 1221, 140201-1405, 1407N, 1408, 1410, 1411, 1508, 160401, 1801, 1802	1											
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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225	155	170	130	90	225	120	540	85	130	85	170	Length of spec. PCR product
	190	190				155						
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
	14											*0418
					18							*0419
						19						*0420
								21				*0423
									22			*0424
										23		*0425
											24	*0426
												*0427
	15											*0428
												*0429
											24	*0430
												*0431
												*0432
												*0433
												*0434
						19						*0435
												*0436
			16			19						*0437
												*010201-0121, 020201-020203, 020205-020206, 0204-0211, 0213-0215, 0217, 0219-0223, 05010101-050104, 0503-0510, 0512-0516, 0518-0523, 06020101-06020102, 060203, 0603, 0606-0619, 0802, 0805, 0807, 0812, 0815, 0817-0819, 120201-1213, 1215-1217, 1221, 140201-1405, 1407N, 1408, 1410, 1411, 1508, 160401, 1801, 1802
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

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Length of spec.	250	220	145	210	250	95	145	270	110	195	180	125
PCR product									180	220		180
									220			
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*0122, 0203, 0216, 0218, 0511, 0517, 0604, 080101-080102, 0803, 0804, 0806, 0808-0811, 0813, 0814, 0816, 1214, 1218, 1220, 1406, 150201-1507, 1509- 1513, 1516-1521, 1701- 1705					5							
*0212	1			4								
*0305, 0325, 0327												
*0334							7					
*0605	1										11	
*0708												
*0764		2					7					
*1409	1											
*1515					5			8				
*1803	1						7					
HLA-Cw allele ⁴												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
B*5802					5			8				
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*04 subtyping. .

In addition, well number 17 contains 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 6th, exon or in the 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, 3rd or 7th 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.

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225	155	170	130	90	225	120	540	85	130	85	170	Length of spec. PCR product
	190	190				155						
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												*0122, 0203, 0216, 0218, 0511, 0517, 0604, 080101-080102, 0803, 0804, 0806, 0808-0811, 0813, 0814, 0816, 1214, 1218, 1220, 1406, 150201-1507, 1509- 1513, 1516-1521, 1701- 1705
												*0212
			16									*0305, 0325, 0327
												*0334
												*0605
	14											*0708
			16									*0764
												*1409
												*1515
												*1803
												HLA-Cw allele ⁴
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												B*5802
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

⁴The nucleotide sequence of the Cw*0402 allele has been shown to be identical to Cw*04010101.

The nucleotide sequence of the Cw*0421 allele has been renamed to Cw*041502.

The nucleotide sequence of the Cw*0422 allele has been shown to be identical to Cw*0421.

⁵Primer mix 9: Specific PCR fragment of 110 bp in the Cw*0435 allele. Specific PCR fragment of 180 bp in the Cw*0430 allele. Specific PCR fragment of 220 bp in the Cw*0409N allele.

Primer mix 10: Specific PCR fragment of 195 bp in the Cw*0431 allele. Specific PCR fragment of 220 bp in the Cw*0410, Cw*0411 and Cw*0436 alleles.

Primer mix 12: Specific PCR fragment of 125 bp in the Cw*0429 and Cw*0436 alleles. Specific PCR fragment of 180 bp in the Cw*0433 allele. Specific PCR fragment of 125 and 180 bp in the Cw*0411 allele.

Primer mix 14: Specific PCR fragment of 155 bp in the Cw*0416 allele. Specific PCR fragment of 190 bp in the Cw*0413, Cw*0418 and Cw*0708 alleles.

Primer mix 15: Specific PCR fragment of 170 bp in the Cw*0414 allele. Specific PCR fragment of 190 bp in the Cw*0428 allele.

Primer mix 19: Specific PCR fragment of 120 bp in the Cw*0435 and Cw*0437 alleles. Specific PCR fragment of 155 bp in the Cw*0420 allele.

'w', may be weakly amplified.

Lot No.: 72F

Lot-specific information

CELL LINE VALIDATION SHEET					HLA-Cw*04 SSP subtyping kit															
					Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
					Prod. No.:															
					200618401	200957902	200618403	200846004	200957905	200618406	200846007	200957908	200957909	200846010	200618411	200957912	200733713	200733714	200846015	200957916
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	9007	DEM	*0602		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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	*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	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-



Lot No.: 72F

Lot-specific information

CELL LINE VALIDATION SHEET					Well							
HLA-Cw*04 SSP subtyping kit					17	18	19	20	21	22	23	24
				Prod. No.:	200957917	200733718	200957919	200846020	200733721	200957922	200733723	200957924
	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	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		*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	-	-	-	-	-	-	-	-

Lot No.: **72F**

Lot-specific information

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-Cw*04 SSP

Product number: 101.612-12u – without *Taq* polymerase
Lot number: 72F
Expiry date: 2011-April-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	2006-184-01	9	2009-539-09	17	2009-539-17
2	2009-539-02	10	2008-460-10	18	2007-337-18
3	2006-184-03	11	2006-184-11	19	2009-539-19
4	2008-460-04	12	2009-539-12	20	2008-460-20
5	2009-539-05	13	2007-337-13	21	2007-337-21
6	2006-184-06	14	2007-337-14	22	2009-539-22
7	2008-460-07	15	2008-460-15	23	2007-337-23
8	2009-539-08	16	2009-539-16	24	2009-539-24

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 6, 8, 10, 12, 13, 15 and 17 to 24 were available. The specificity of the primers in primer solutions 8, 10, 12, 15, 17, 20, 21 and 24 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 6, 13 and 18 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 22 and 24 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 15 and 19 one of the 5'-primers was not possible to test, and in primer solutions 10 and 14 one of the 3'-primers was not possible to test. In primer solutions 9 and 24 one 5'-primer and one 3'-primer was not possible to test. Additional primers in primer solutions 7, 9, 14 and 16 were tested by separately adding one 5'-primer and/or one 3'-primer.

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

Date of approval: 2009-May-25

Approved by:

Quality Control, Supervisor

Lot No.: **72F**

Lot-specific information

Declaration of Conformity

Product name: *Olerup* SSP® HLA-Cw*04
Product number: 101.612-12u
Lot number: 72F

Intended use: HLA-Cw*04 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 9001:2003, ISO 17025:1999 and ISO 13485:2003, 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.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

Saltsjöbaden, Sweden
2009-May-25

Olle Olerup
Managing Director

Lot No.: **72F**

Lot-specific information

Lot No.: **72F**

Lot-specific information

Lot No.: **72F**

Lot-specific information

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