

Lot No.: **32F**

Lot-specific information

Olerup SSP[®] HLA-Cw*14

Product number: 101.625-06u – without *Taq* polymerase
Lot number: 32F
Expiry date: 2010-November-01
Number of tests: 6
Number of wells per test: 14
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. 32F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP[®]* HLA-Cw*14 LOT

The HLA-Cw*14 specificity and interpretation tables have been updated for the HLA-Cw alleles described since the previous *Olerup SSP[®]* HLA-Cw*14 lot was made (**Lot No. Y09**).

Two wells have been added to the HLA-Cw*14 kit,
wells **13 and 14**.

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

Well	5'-primer	3'-primer	rationale
13	New	New	New primer pair for the Cw*1410 allele
14	New	New	New primer pair for the Cw*1411 allele

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PRODUCT DESCRIPTION

HLA-Cw*14 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the Cw*1402 to Cw*1411 alleles.

PLATE LAYOUT

Each HLA-Cw*14 test consists of 14 PCR reactions in a 16 well cut PCR plate. Wells 15 to 16 are empty

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

The 16 well cut PCR plate is marked with 'HLA-Cw*14' in silver/gray ink .

Well No. 1 is marked with the Lot No. '32F'.

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*14 SSP subtypings will be influenced by other HLA-Cw alleles, as primer mixes 1 to 9 amplifies non-HLA-Cw*14 alleles. In addition, primer mix 4 will amplify the B*350802 and B*6702 alleles, primer mix 5 will amplify the A*2487 and B*1584 alleles, primer mix 7 will amplify the B*0713 and B*0716 alleles and primer mix 8 will amplify the B*5530 allele.

UNIQUELY IDENTIFIED ALLELES

All the HLA-Cw*14 alleles, i.e. **Cw*1402 to Cw*1411**, recognized by the HLA Nomenclature Committee in October 2008¹ will be amplified by the primers in the HLA-Cw*14 SSP kit.

The HLA-Cw*14 subtyping kit cannot distinguish the Cw*140201 and Cw*140204 alleles.

¹HLA-Cw alleles listed on the IMGT/HLA web page 2008-October-10, release 2.23.0, www.ebi.ac.uk/imgt/hla.

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RESOLUTION IN HOMO- AND HETEROZYGOTES

The ten phenotypically different HLA-Cw*14 alleles give rise to 12 amplification patterns that can be combined in 78 homozygous and heterozygous combinations. Forty-nine of these genotypes do not give rise to unique amplification patterns.

+++---+-	+-----	1405,1410 = 1409,1410
+++---+-	+-----	1403,1405 = 1403,1409
+++---+-	+-----	1405,1408 = 1408,1409
+++---+-	+-----	140203,1405 = 140203,1409
+++---+-	+-----	1402,1410 = 140202,1410
+++---+-	+-----	1402,1403 = 140202,1403
+++---+-	+-----	1402,1408 = 140202,1408
+++---+-	+-----	1402,140203 = 140202,140203
+++---+-	+-----	1404,1405 = 1404,1409
+++---+-	+-----	1402,1404 = 140202,1404 = 1404,1404
+++---+-	+-----	1405,1407N = 1407N,1409
+++---+-	+-----	1405,1411 = 1409,1411
+++---+-	+-----	1402,1405 = 1402,1409 = 140202,1405
+++---+-	+-----	1402,1406 = 140202,1406
+++---+-	+-----	1402,1407N = 140202,1407N = 1407N,1407N
+++---+-	+-----	1402,1411 = 140202,1411 = 1411,1411
+++---+-	+-----	1402,1402 = 1402,140202
+++---+-	+-----	1405,1406 = 1406,1409
+++---+-	+-----	1405,1405 = 1405,1409
+++---+-	+-----	140203,1410 = 1403,1410 = 1410,1410
+++---+-	+-----	140203,1403 = 1403,1403
+++---+-	+-----	140203,1408 = 1408,1408

1402 = 140201 and 140204

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SPECIFICITY TABLE

HLA-Cw*14 SSP subtyping

Specificities and sizes of the PCR products of the 14 primer mixes used for HLA-Cw*14 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-Cw*14 alleles	Other amplified HLA Class I alleles ³
1	145 bp	800 bp	140201-140202, 140204, 1404-1407N, 1409, 1411	0764
2⁵	145 bp	1070 bp	140203, 1403, 1408, 1410	04010101-040106, 040401-0405, 0407-041502, 0417-0420, 0423-0436
3	245 bp	800 bp	1404	04010101-040106, 040401-0405, 0407-0410, 0412-041502, 0417-0420, 0423-0425, 0426 ^{weakly} , 0427, 0428, 0430-0435
4	140 bp	1070 bp	140203, 1403, 1408, 1410	0121, 0212 ^{weakly} , 0411, 0429, 0436, 080101-0809, 0811-0819, 120201-120303, 120305, 120306, 1206-1208, 1210-1220, 1507, 1521 ^{weakly} , 160101, 160103, 160401, 1606-1608, 1610, 1611, B*350802, B*6702
5	210 bp	1070 bp	140201-1404, 1407N, 1408, 1410, 1411	04010101-040106, 0403, 0405, 0407-0412, 0416, 0418-0420, 0423-0433, 0435, 0436, 0819, 1801, 1802, A*2487, B*1584
6⁴	130 bp	1070 bp	1405, 1409	030201-030407, 0306-0313, 0315-0324, 0326, 0328-0350, 041501-

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7⁴	130 bp	1070 bp	1404	041502, 0417 0117, 0315, 0327, 033801-033802, 06020101-06020102, 060203, 0603, 0604, 0606-0618, 070101- 0720, 0722-0733, 0735-0738, 0741- 0764, 120304, 120401, 1701-1704, 1801-1803, B*0713, B*0715
8	210 bp	1070 bp	1403, 1410	0212 ^{weakly} , 030201- 0306, 0308, 0309, 0310 ^{weakly} , 0312- 0314, 0316, 0317, 0319-0328, 0329 ^{weakly} , 0330-0336, 033801- 033802, 0340-0344, 0346-0349, 0720, 1507, 1521 ^{weakly} , B*5530
9	140 bp	1070 bp	140201, 140203-1408, 1410, 1411	0317
10	210 bp	1070 bp	1406	
11	205 bp	1070 bp	1407N	
12	225 bp	1070 bp	1408	
13	285 bp	800 bp	1410	
14	290 bp	1070 bp	1411	

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*14 resolution 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 is 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

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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*14 SSP subtyping.

In addition, wells number 3 and 13 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.

³Due to the sharing of sequence motifs between HLA-Cw alleles some non-HLA-Cw*14 alleles will be amplified by primer mixes 2 to 9. In addition, primer mix 4 will amplify the B*350802 and B*6702 alleles, primer mix 5 will amplify the A*2487 and B*1584 alleles, primer mix 7 will amplify the B*0713 and B*0716 alleles and primer mix 8 will amplify the B*5530 allele.

⁴Specific PCR fragments shorter than 130 base pairs have a lower intensity and are less sharp than longer PCR bands.

⁵Primer mix 2 has a tendency of giving rise to nonspecific amplifications.

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INTERPRETATION TABLE								
HLA-Cw*14 SSP subtyping								
Amplification patterns of the HLA-Cw*1402 to 1411 alleles								
	Well							
	1	2	3	4	5	6	7	8
Length of spec.	145	145	245	140	210	130	130	210
PCR product								
Length of int.	800	1070	800	1070	1070	1070	1070	1070
pos. control¹								
5'-primer²	98	98	98	201	368	368	201	134
	5'-CTC ^{3'}	5'-CTC ^{3'}	5'-CTC ^{3'}	5'-CCA ^{3'}	5'-gTT ^{3'}	5'-gTA ^{3'}	5'-CCg ^{3'}	5'-CCA ^{3'}
3'-primer³	201	201	302	302	538	459	289	302
	5'-CTC ^{3'}	5'-CTT ^{3'}	5'-ggT ^{3'}	5'-ggC ^{3'}	5'-CCg ^{3'}	5'-AgA ^{3'}	5'-AgC ^{3'}	5'-ggC ^{3'}
Well No.	1	2	3	4	5	6	7	8
HLA-Cw allele⁴								
*140201, 140204	1				5			
*140202	1				5			
*140203		2		4	5			
*1403		2		4	5			8
*1404	1		3		5		7	
*1405	1					6		
*1406	1							
*1407N	1				5			
*1408		2		4	5			
*1409	1					6		
*1410		2		4	5			8
*1411	1				5			
*0117, 06020101-06020102, 060203, 0603, 0604, 0606-0618, 070101-0719, 0722-0733, 0735-0738, 0741-0763, 120304, 120401, 1701-1704, 1803							7	
*0121, 080101-0809, 0811-0818, 120201-120303, 120305, 120306, 1206-1208, 1210-1220, 160101, 160103, 160401, 1606-1608, 1610, 1611				4				
*0212, 1521				w				w
Well No.	1	2	3	4	5	6	7	8

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INTERPRETATION TABLE						
HLA-Cw*14 SSP subtyping						
Amplification patterns of the HLA-Cw*1402 to 1411 alleles						
Well						
9	10	11	12	13	14	
140	210	205	225	285	290	Length of spec.
						PCR product
1070	1070	1070	1070	800	1070	Length of int.
						pos. control¹
361	368	419	356	98	368	5'-primer²
5'-AgT ^{3'}	5'-gTT ^{3'}	5'-gTC ^{3'}	5'-CCC ^{3'}	5'-CTC ^{3'}	5'-gTT ^{3'}	
459	539	585	538	343	619	3'-primer³
5'-AgA ^{3'}	5'-TCA ^{3'}	5'-AgT ^{3'}	5'-CCg ^{3'}	5'-ACT ^{3'}	5'-ACT ^{3'}	
9	10	11	12	13	14	Well No.
						HLA-Cw allele⁴
9						*140201, 140204
						*140202
9						*140203
9						*1403
9						*1404
9						*1405
9	10					*1406
9		11				*1407N
9			12			*1408
						*1409
9				13		*1410
9					14	*1411
						*0117, 06020101-06020102, 060203, 0603, 0604, 0606-0618, 070101-0719, 0722-0733, 0735-0738, 0741-0763, 120304, 120401, 1701-1704, 1803
						*0121, 080101-0809, 0811-0818, 120201-120303, 120305, 120306, 1206-1208, 1210-1220, 160101, 160103, 160401, 1606-1608, 1610, 1611
						*0212, 1521
9	10	11	12	13	14	Well No.

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Length of spec.	145	145	245	140	210	130	130	210
PCR product								
Well No.	1	2	3	4	5	6	7	8
*030201-030407, 0306, 0308, 0309, 0312, 0313, 0316, 0319-0324, 0326, 0328, 0330-0336, 0340-0344, 0346-0349						6		8
*0305, 0314, 0325								8
*0307, 031101-031102, 0318, 0337, 0339, 0345, 0350						6		
*0310, 0329						6		w
*0315						6	7	
*0317						6		8
*0327, 0720							7	8
*033801-033802						6	7	8
*04010101-040106, 0405, 0407-0410, 0412, 0418-0420, 0423-0425, 0427, 0428, 0430-0433, 0435		2	3		5			
*0403, 0416					5			
*040401-040402, 0413, 0414, 0434		2	3					
*0411, 0429, 0436		2		4	5			
*041501-041502, 0417		2	3			6		
*0426		2	w		5			
*0764	1						7	
*0819				4	5			
*1507				4				8
*1801, 1802					5		7	
HLA-Cw allele ⁴								
Well No.	1	2	3	4	5	6	7	8
A*2487, B*1584					5			
B*0713, B*0715							7	
B*350802, B*6702				4				
B*5530								8
Well No.	1	2	3	4	5	6	7	8

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140	210	205	225	285	290	Length of spec. PCR product
9	10	11	12	13	14	Well No.
						*030201-030407, 0306, 0308, 0309, 0312, 0313, 0316, 0319-0324, 0326, 0328, 0330-0336, 0340- 0344, 0346-0349
						*0305, 0314, 0325
						*0307, 031101-031102, 0318, 0337, 0339, 0345, 0350
						*0310, 0329
						*0315
9						*0317
						*0327, 0720
						*033801-033802
						*04010101-040106, 0405, 0407-0410, 0412, 0418- 0420, 0423-0425, 0427, 0428, 0430-0433, 0435
						*0403, 0416
						*040401-040402, 0413, 0414, 0434
						*0411, 0429, 0436
						*041501-041502, 0417
						*0426
						*0764
						*0819
						*1507
						*1801, 1802
						HLA-Cw allele ⁴
9	10	11	12	13	14	Well No.
						A*2487, B*1584
						B*0713, B*0715
						B*350802, B*6702
						B*5530
9	10	11	12	13	14	Well No.

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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*14 SSP subtyping.

In addition, wells number 3 and 13 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 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.

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. 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*1401 nucleotide sequence has been shown to be identical to Cw*1402.

'w', may be weakly amplified.

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CELL LINE VALIDATION SHEET																		
HLA-Cw*14 SSP primer set																		
				Well														
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	
				Prod. No.:														
				200853501	200853502	200853503	200853504	200853505	200853506	200853507	200853508	200853509	200853510	200853511	200853512	200853513	200853514	
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	-	+	+	+	+	-	-	-	-	-	-	-	-	

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CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-Cw*14 SSP

Product number: 101.625-06u – without *Taq* polymerase
Lot number: 32F
Expiry date: 2010-November-01
Number of tests: 6
Number of wells per test: 14

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2008-535-01	9	2008-535-09
2	2008-535-02	10	2008-535-10
3	2008-535-03	11	2008-535-11
4	2008-535-04	12	2008-535-12
5	2008-535-05	13	2008-535-13
6	2008-535-06	14	2008-535-14
7	2008-535-07		
8	2008-535-08		

The specificity of each primer solution of the HLA-Cw*14 primer set has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer mixes 10 to 14 were available. The specificities of the primers in primer solutions 10 and 13 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 11 and 14, it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solution 12, it was only possible to test the 3'-primer, the 5'-primer was not possible to test. Finally, one of the 5'-primers in primer solution 6 was tested by adding one additional 3'-primer.

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

Date of approval: 2009-May-26

Approved by:

Quality Control, Supervisor

Lot No.: **32F**

Lot-specific information

Declaration of Conformity

Product name: *Olerup* SSP® HLA-Cw*14
Product number: 101.625-06u
Lot number: 32F

Intended use: HLA-Cw*14 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
2009-May-26

Olle Olerup
Managing Director

Lot No.: **32F**

Lot-specific information

ADDRESSES:

Manufacturer:

Olerup SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

Tel: +46-8-717 88 27

Fax: +46-8-717 88 18

E-mail: info-ssp@olerup.com

Web page: <http://www.olerup.com>

Distributed by:

Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.

Tel: +43-1-710 15 00

Fax: +43-1-710 15 00 10

E-mail: support-at@olerup.com

Web page: <http://www.olerup.com>

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382

Tel: 1-877-OLERUP1

Fax: 610-344-7989

E-mail: info.us@olerup.com

Web page: <http://www.olerup.com>

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