

Olerup SSP[®] HLA-Cw*06

Product number:	101.614-12 – including <i>Taq</i> polymerase 101.614-12u – without <i>Taq</i> polymerase
Lot number:	01F
Expiry date:	2010-July-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. 01F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] HLA-Cw*06 LOT

The HLA-Cw*06 specificity and interpretation tables have been updated for the HLA-Cw alleles described since the previous *Olerup SSP*[®] HLA-Cw*06 lot was made (**Lot No. X85**).

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

Well	5'-primer	3'-primer	rationale
9	Added	Added	Primer pair added for the Cw*0617 allele.

PRODUCT DESCRIPTION

HLA-Cw*06 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-Cw*0602 to HLA-Cw*0617 alleles.

PLATE LAYOUT

Each test consists of 16 PCR reactions in a 16 well PCR plate.

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

The 16 well cut PCR plate is marked with 'Cw*06'.

Well No. 1 is marked with the Lot No. '01F'.

The PCR plates are covered with a PCR-compatible foil.

Please note: When removing each 16 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

The interpretation of HLA-Cw*06 SSP subtypings will be influenced by the Cw*01 alleles, two Cw*02 alleles, the Cw*03 alleles, most Cw*04 alleles, the Cw*05 alleles, the Cw*08 alleles, most Cw*12 alleles, the Cw*14 alleles, the Cw*15 alleles, the Cw*160401 allele, the Cw*17 alleles and the Cw*18 alleles when present on the other haplotype. In addition, the B*5802 allele will be amplified by primer mix 4.

UNIQUELY IDENTIFIED ALLELES

All the HLA-C*06 alleles, i.e. **Cw*0602 to Cw*0617**, recognized by the HLA Nomenclature Committee in April 2008¹ will give rise to unique amplification patterns by the primers in the HLA-Cw*06 subtyping kit.

The HLA-Cw*06 SSP subtyping kit cannot distinguish the Cw*06020101, Cw*06020102 and Cw*060203 alleles.

¹HLA-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 16 HLA-Cw*06 alleles can be combined in 136 homozygous and heterozygous combinations. Forty-six of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products generated by primer mix 9 was not considered in these calculations.

+++-----	+-----	0604,0609 = 0604,0617
+++--+-	+-----	0605,0609 = 0605,0617
+++---+	+-----	0606,0609 = 0606,0617
+++----+	+-----	0607,0609 = 0607,0617
+++-----	-----	0602,0607 = 0607,0607
+++-----	+-----	0608,0609 = 0608,0617
+++-----	-----	0602,0608 = 0608,0608
+++-----	++-----	0609,0610 = 0610,0617
+++-----	+--+-	0609,0611 = 0611,0617
+++-----	+--+---	0609,0612 = 0612,0617
+++-----	+---+---	0609,0613 = 0613,0617
+++-----	+----+-	0609,0614 = 0614,0617
+++-----	+-----+	0609,0615 = 0615,0617
+++-----	+-----+	0609,0616N = 0616N,0617
+++-----	+-----	0602,0609 = 0602,0617 = 0609,0609 = 0609,0617
+++-----	-+-----	0602,0610 = 0610,0610
+++-----	--+-----	0602,0611 = 0611,0611
+++-----	----+-----	0602,0612 = 0612,0612
+++-----	-----+---	0602,0613 = 0613,0613
+++-----	-----+-	0602,0615 = 0615,0615
+++-----	-----+	0602,0616N = 0616N,0616N
+---+-	-----	0605,0605 = 0605,0606

SPECIFICITY TABLE

HLA-Cw*06 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-Cw*06 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-Cw*06 alleles	Other amplified HLA Class I alleles ³
1	240 bp	800 bp	06020101-06020102, 060203, 0604-0616N	0339, 1216
2	220 bp	800 bp	06020101-06020102, 060203, 0603, 0607-0613, 0615-0617	0104, 0109, 0205, 0217, 12030101-1207, 1211-1213, 1215, 160401
3 ⁴	135 bp	1070 bp	0603	030201-0317, 0319-033802, 0340-0348
4	240 bp	1070 bp	0604	0511, 0517, 080101-080102, 0803, 0804, 0806, 0808-0811, 0813, 0814, 0816, 1214, 1218, 1220, 1406, 150201-1507, 1509-1521, 1701-1704, B*5802
5	165 bp	800 bp	0605	050101-050104, 0503-0519, 0810, 1221
6	240 bp	1070 bp	0605, 0606	010201-0103, 0105-0108, 0110-0120, 04010101-040105, 0403, 0405, 0407-0412, 041501-0420, 0423-0432, 050101-0507N, 0509, 0510, 0512-0516, 0518, 0519, 0802, 0805, 0807, 0812, 0815, 1209, 140201-1405, 1407N-1409, 1508, 1801, 1802
7 ⁴	110 bp	1070 bp	0607	
8	240 bp	1070 bp	0608	0110, 0205, 0217

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9⁵	165, 435 bp	1070 bp	0609, 0617	0508, 0707, 0709, 1801-1803
10	200 bp	800 bp	0610	
11⁴	130 bp	1070 bp	0611	070401-070402, 0711, 0712, 0745
12	210 bp	1070 bp	0612	
13	155 bp	1070 bp	0613	
14	295 bp	1070 bp	0614	0332, 0345, 0710, 0743
15	365 bp	800 bp	0615	
16	240 bp	1070 bp	0616N	

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*06 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 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*06 subtyping.

In addition, wells number 2, 5, 10 and 15 contain the primer pair giving rise to the shorter, 800 bp, internal positive control 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 non-HLA-Cw*06 alleles will be amplified by primer mixes 1 to 6, 8, 9, 11 and 14. In addition, the B*5802 allele will be amplified by primer mix 4.

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

⁵Primer mix 9: Specific PCR fragment of 165 bp in the Cw*0609 and Cw*0508 alleles. Specific PCR fragment of 435 bp in the Cw*0617 and Cw*0707, 0709 and 1801 to 1803 alleles.

INTERPRETATION TABLE								
HLA-Cw*06 SSP subtyping								
Amplification patterns of the Cw*0602 to Cw*0617 alleles								
	Well⁵							
	1	2	3	4	5	6	7	8
Length of spec.	240	220	135	240	165	240	110	240
PCR product(s)								
Length of int.	800	800	1070	1070	800	1070	1070	1070
pos. control¹								
5'-primer(s)²	28	361	105	2nd In	176	2nd In	232	361
	5'-TCA ^{3'}	5'-AgT ^{3'}	5'-gCT ^{3'}	5'-CCA ^{3'}	5'-gCA ^{3'}	5'-CCA ^{3'}	5'-AgA ^{3'}	5'-AgT ^{3'}
3'-primer(s)³	97	538	201	539	302	538	302	539
	5'-gTC ^{3'}	5'-CCA ^{3'}	5'-CTC ^{3'}	5'-TCA ^{3'}	5'-ggT ^{3'}	5'-CCg ^{3'}	5'-ggT ^{3'}	5'-CTC ^{3'}
Well No.	1	2	3	4	5	6	7	8
HLA-Cw allele⁴								
*06020101-06020102, 060203	1	2						
*0603		2	3					
*0604	1			4				
*0605	1				5	6		
*0606	1					6		
*0607	1	2					7	
*0608	1	2						8
*0609	1	2						
*0610	1	2						
*0611	1	2						
*0612	1	2						
*0613	1	2						
*0614	1							
*0615	1	2						
*0616N	1	2						
*0617		2						
*010201-0103, 0105-0108, 0111-0120, 04010101-040105, 0403, 0405, 0407-0412, 041501-0421, 0423-0432, 0802, 0805, 0807, 0812, 0815, 1209, 140201-1405, 1407N-1409, 1508						6		
Well No.	1	2	3	4	5	6	7	8

INTERPRETATION TABLE								
HLA-Cw*06 SSP subtyping								
Amplification patterns of the Cw*0602 to 0617 alleles								
Well ⁵								
9	10	11	12	13	14	15	16	
165	200	130	210	155	295	365	240	Length of spec.
435								PCR product(s)
1070	800	1070	1070	1070	1070	800	1070	Length of int. pos. control ¹
47	142	213	529	361	341	376	501	5'-primer(s) ²
5'-Agg ^{3'}	5'-TCC ^{3'}	5'-CCC ^{3'}	5'-AgA ^{3'}	5'-AgT ^{3'}	5'-ggA ^{3'}	5'-gCT ^{3'}	5'-ggC ^{3'}	
412								
5'-ATA ^{3'}								
312	302	302	3 rd I	475	353	3 rd I	3 rd I	3'-primer(s) ³
5'-AgT ^{3'}	5'-ggT ^{3'}	5'-ggC ^{3'}	5'-CTC ^{3'}	5'-ggT ^{3'}	5'-TgA ^{3'}	5'-CTC ^{3'}	5'-CTC ^{3'}	
538								
5'-CCA ^{3'}								
9	10	11	12	13	14	15	16	Well No.
								HLA-Cw allele ⁴
								*06020101-06020102, 060203
								*0603
								*0604
								*0605
								*0606
								*0607
								*0608
9								*0609
	10							*0610
		11						*0611
			12					*0612
				13				*0613
					14			*0614
						15		*0615
							16	*0616N
9								*0617
								*010201-0103, 0105-0108, 0111-0120, 04010101-040105, 0403, 0405, 0407-0412, 041501-0421, 0423-0432, 0802, 0805, 0807, 0812, 0815, 1209, 140201-1405, 1407N-1409, 1508
9	10	11	12	13	14	15	16	Well No.

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Lot-specific information

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Length of spec.	240	220	135	240	165	240	110	240
PCR product(s)								
Well No.	1	2	3	4	5	6	7	8
*0104, 0109, 12030101-1207, 1211-1213, 1215, 160401	2							
*0110						6		8
*0205, 0217	2		8					
*030201-0317, 0319-0331, 0333-033802, 0340-0344, 0346-0348			3					
*0332, 0345	3							
*0339, 1216	1							
*050101-050104, 0503-0507N, 0509, 0510, 0512-0516, 0518, 0519					5	6		
*0508					5			
*0511, 0517, 0810				4	5			
*070401-070402, 0711, 0712, 0745								
*0707, 0709, 1803								
*0710, 0743								
*080101-080102, 0803, 0804, 0806, 0808, 0809, 0811, 0813, 0814, 0816, 1214, 1218, 1220, 1406, 150201-1507, 1509-1521, 1701-1704				4				
*1221					5			
*1801, 1802					6			
HLA-Cw allele ⁴								
Well No.	1	2	3	4	5	6	7	8
B*5802				4				
Well No.	1	2	3	4	5	6	7	8

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-Cw*06 subtyping.

In addition, wells number 2, 5, 10 and 15 contain the primer pair giving rise to the shorter, 800 bp, internal positive control in order to allow kit identification.

²The nucleotide position, in the 1st, 2nd or 3rd exons or the 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 or 3rd exons 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.

Lot No.: **01F**

Lot-specific information

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165	200	130	210	155	295	365	240	Length of spec.
435								PCR product(s)
9	10	11	12	13	14	15	16	Well No.
								*0104, 0109, 12030101-1207, 1211-1213, 1215, 160401
								*0110
								*0205, 0217
								*030201-0317, 0319-0331, 0333-033802, 0340-0344, 0346-0348
				14				*0332, 0345
								*0339, 1216
								*050101-050104, 0503-0507N, 0509, 0510, 0512-0516, 0518, 0519
9								*0508
		11						*0511, 0517, 0810
9								*070401-070402, 0711, 0712, 0745
					14			*0707, 0709, 1803
								*0710, 0743
								*080101-080102, 0803, 0804, 0806, 0808, 0809, 0811, 0813, 0814, 0816, 1214, 1218, 1220, 1406, 150201-1507, 1509-1521, 1701-1704
								*1221
9								*1801, 1802
								HLA-Cw allele ⁴
9	10	11	12	13	14	15	16	Well No.
								B*5802
9	10	11	12	13	14	15	16	Well No.

⁴The sequence of the Cw*0601 allele has been shown to be identical to Cw*0602.

The sequence of the Cw*060202 allele has been renamed to Cw*0617.

⁵Primer mix 9: Specific PCR fragment of 165 bp in the Cw*0609 and Cw*0508 alleles. Specific PCR fragment of 435 bp in the Cw*0617 and Cw*0707, 0709 and 1801 to 1803 alleles.

CELL LINE VALIDATION SHEET																			
HLA-Cw*06 SSP subtyping kit																			
				Well															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.:															
				200734401	200734402	200734403	200734404	200734405	200734406	200734407	200850409	200734409	200734410	200734411	200734412	200734413	200734414	200734415	200734416
IHWc cell line		Cw*																	
1	9001	SA	*0702	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*0701	*1505	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*1202		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*0304	*0401	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*0602		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*0304	*0702	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*0501		-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
8	9007	DEM	*0602		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*1203		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*0102		-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0602		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9067	BTB	*0102		-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*0102	*0304	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
15	9075	DKB	*0304		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*0202		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9008	WILJON	*1203		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*0102	*0705	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
19	9038	BM16	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*0304		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*0303		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*1203		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*0102	*1502	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*1203		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*0802		-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
26	9285	WT49	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*0704	*1505	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
28	9320	BEL5GB	*0501	*1601	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
29	9050	MOU	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*1701		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*0501		-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
32	9297	HAG	*1701	*1703	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*0304		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*1203		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*0501		-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
36	9024	KT17	*0303	*0401	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*0303		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*0202	*0701	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*0602		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*0802		-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*0102		-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
43	9076	T7526	*0102	*0801	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
44	9057	TEM	*1203		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*0602	*17	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*0704	*1502	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
48	9303	TER-ND	*0401	*1601	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-



CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-Cw*06 SSP

Product number: 101.614-12 – including *Taq* polymerase
101.614-12u – without *Taq* polymerase
Lot number: 01F
Expiry date: 2010-July-01
Number of tests: 12
Number of wells per test: 16

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2007-344-01	9	2008-504-09
2	2007-344-02	10	2007-344-10
3	2007-344-03	11	2007-344-11
4	2007-344-04	12	2007-344-12
5	2007-344-05	13	2007-344-13
6	2007-344-06	14	2007-344-14
7	2007-344-07	15	2007-344-15
8	2007-344-08	16	2007-344-16

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 7, 8, 10, 12 to 16 were available. It was only possible to test the 3'-primer in primer solutions 7, 10, 12, 15 and 16 by separately adding another 5'-primer, the 5'-primer was not possible to test. In primer solutions 8 and 14 it was possible to test both the 5'-primer and the 3'-primer. In primer solution 13 it was only possible to test the 5'-primer, the 3'-primer was not possible to test.

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

Date of approval: 2008-July-15

Approved by:

Quality Control, Supervisor

Lot No.: **01F**

Lot-specific information

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Declaration of Conformity

Product name: *Olerup* SSP[®] HLA-Cw*06
Product number: 101.614-12, 101.614-12u
Lot number: 01F

Intended use: HLA-Cw*06 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, 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.

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

Saltsjöbaden, Sweden
2008-July-15

Olle Olerup

HLA-Cw*06
101.614-12 – including *Taq* polymerase
101.614-12u – without *Taq* polymerase

Product Insert

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Lot No.: **01F**

Lot-specific information

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Lot-specific information

www.olerup.com

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**.