

Lot No.: **80E**

Lot-specific information

Olerup SSP[®] HLA-A*33

Product number: 101.432-12u – without *Taq* polymerase
Lot number: 80E
Expiry date: 2010-June-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. 80E.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP[®]* HLA-A*33 LOT

The HLA-A*33 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP[®]* HLA-A*33 lot was made (Lot No. X90).

Five wells have been added to the HLA-A*33 kit,
wells **12 to 16**.

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

Well	5'-primer	3'-primer	rationale
6	-	Modified	Increased specificity of specific primer pair.
9	-	Added	Primer added for the A*3314 allele.
10	-	Added	Primer added for the A*3313 allele.
12	New	New	New primer pair for the A*3311 allele.
13	New	New	New primer pair for the A*3312 allele.
14	New	New	New primer pair for the A*3315 allele.
15	New	New	New primer pair for the A*3316 allele.
16	New	New	New primer pair for the A*3317 allele.

Changes in revision R02 compared to R01:

1. In the Interpretation and Specificity Tables the control band size in well 6 has been corrected to 1070 bp.

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PRODUCT DESCRIPTION

HLA-A*33 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*3301 to A*3317 alleles.

PLATE LAYOUT

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

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

The 16 well cut PCR plate is marked with 'HLA-A*33'.

Well No. 1 is marked with the Lot No. '80E'.

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-A*33 SSP subtypings will be influenced by six A*02, one A*23, two A*24, the A*25, the A*26, most A*31, the A*32, three A*34, the A*4301, most A*66, A*6829 and the A*74 alleles when present on the other haplotype.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*33 alleles, i.e. **A*3301 to A*3317 alleles**, recognized by the HLA Nomenclature Committee in April 2008¹ will give rise to unique amplification patterns by the primers in the HLA-A*33 subtyping kit.

The A*33 subtyping kit cannot distinguish the A*330301 and A*330302 alleles.

¹HLA-A 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-A*33 alleles can be combined in 136 homozygous and heterozygous combinations. Fifty-three of these genotypes do not give rise to unique amplification patterns. The different sizes of the specific PCR products generated by primer mixes 9 and 10 were not considered in this calculation.

+++++---	+-----	3305, 3309 = 3305, 3314
++++----	+-----	3307, 3309 = 3307, 3314
++++-----	+-----+	3309, 3316 = 3314, 3316
++++-----	+-----	3301, 3309 = 3301, 3314
+++---+	-----	3301, 3305 = 3305, 3305
+++-----	-----	3301, 3307 = 3307, 3307
+++-----	-----+	3301, 3316 = 3316, 3316

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++++-----	+-----	3304,3309 = 3304,3314
+---+---+	+-----	3306,3309 = 3306,3314
+---+---+	-----	3303,3306 = 3306,3306
+---+-----	++-----	3303,3308 = 3308,3313 = 3308,3314 = 3309,3313 = 3313,3314
+---+-----	+---+-----	3309,3310 = 3310,3314
+---+-----	+---+-----	3309,3311 = 3311,3314
+---+-----	+---+-----	3309,3312 = 3312,3314
+---+-----	+-----+--	3309,3315 = 3314,3315
+---+-----	+-----++	3309,3317 = 3314,3317
+---+-----	+-----	3303,3309 = 3303,3314 = 3309,3314 = 3314,3314
+---+-----	-+-----	3303,3313 = 3313,3313
+---+-----	--+-----	3303,3310 = 3310,3310
+---+-----	----+-----	3303,3311 = 3311,3311
+---+-----	-----+---	3303,3312 = 3312,3312
+---+-----	-----+--	3303,3315 = 3315,3315
+---+-----	-----++	3303,3317 = 3317,3317
---+-----	++-----	3308,3308 = 3308,3309

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SPECIFICITY TABLE

HLA-A*33 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-A*33 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*33 alleles	Other amplified HLA-A alleles ³
1	205 bp	800 bp	3301, 330301-3307, 3310-3317	6829
2	205 bp	800 bp	3301, 3304, 3305, 3307, 3316	6604
3	155 bp	1070 bp	3301, 3305, 3307, 3316	6604
4	210 bp	1070 bp	330301-330302, 3306, 3308-3315, 3317	0241, 0265, 0280, 9217, 9235, 2462, 250101-2506, 260101-2635, 310102-310203, 3102, 3105, 3107-3122, 320101-3203, 3205-3216, 340101-340102, 3405, 3406, 4301, 6601-6603, 6605-6607, 7401-7412N
5⁴	90 bp	800 bp	3304	
6⁵	175 bp	1070 bp	3305	
7⁴	105 bp	1070 bp	3306	
8⁴	125 bp	1070 bp	3307	
9⁶	160, 185 bp	1070 bp	3308, 3309, 3314	
10⁷	215, 285 bp	1070 bp	3308, 3313	2482, 3102, 3103, 3107, 3108
11	165 bp	1070 bp	3310	230301, 2903, 3105, 3213
12	235 bp	1070 bp	3311	6829
13⁴	95 bp	1070 bp	3312	
14⁴	115 bp	1070 bp	3315	9240
15	140 bp	1070 bp	3316	
16	245 bp	1070 bp	3317	

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¹ Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of HLA-A*33 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.

² The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-A*33 subtyping.

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

In the presence of a specific amplification the intensity of the control band often decreases.

³ Due to the sharing of sequence motifs between HLA-A alleles non-HLA-A*33 alleles will be amplified by primer mixes 1, 2, 3, 4, 10 and 11.

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

⁵ Primer mix 6 may give to non-specific amplifications.

⁶ Primer mix 9: Specific PCR fragment of 160 bp in the A*3314 allele. Specific PCR fragment of 185 bp in the A*3308 and 3309 alleles.

⁷ Primer mix 10: Specific PCR fragment of 215 bp in the A*3308 and A*2482, 3102, 3107 and 3108 alleles. Specific PCR fragment of 285 bp in the A*3313 and A*3103 alleles.

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Lot-specific information

INTERPRETATION TABLE								
HLA-A*33 SSP subtyping								
Amplification patterns of the A*3301 to A*3317 alleles								
	Well⁵							
	1	2	3	4	5	6	7	8
Length of spec.	205	205	155	210	90	175	105	125
PCR product(s)								
Length of int.	800	800	1070	1070	800	1070	1070	1070
pos. control¹								
5'-primer²	97	418	468	414	414	97	228	448
	5'-TCA ^{3'}	5'-Agg ^{3'}	5'-TCT ^{3'}	5'-CAg ^{3'}	5'-CAg ^{3'}	5'-TCA ^{3'}	5'-ATg ^{3'}	5'-CCT ^{3'}
3'-primer(s)³	259	583	583	583	463	233	290	530
	5'-gT ^{3'}	5'-gTg ^{3'}	5'-gTg ^{3'}	5'-gTA ^{3'}	5'-gCT ^{3'}	5'-CCC ^{3'}	5'-CAA ^{3'}	5'-CCT ^{3'}
Well No.	1	2	3	4	5	6	7	8
HLA-A allele⁴								
*3301	1	2	3					
*330301-330302	1			4				
*3304	1	2			5			
*3305	1	2	3			6		
*3306	1			4			7	
*3307	1	2	3					8
*3308				4				
*3309				4				
*3310	1			4				
*3311	1			4				
*3312	1			4				
*3313	1			4				
*3314	1			4				
*3315	1			4				
*3316	1	2	3					
*3317	1			4				
*0241, 0265, 0280, 9217, 9235, 2462, 250101-2506, 260101-2635, 310102-310203, 3109-3122, 320101-3203, 3205-3212, 3214-3216, 340101-3340102, 3405, 3406, 4301, 6601-6603, 6605-6607, 7401-7412N				4				
*9240								
Well No.	1	2	3	4	5	6	7	8

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INTERPRETATION TABLE								
HLA-A*33 SSP subtyping								
Amplification patterns of the A*3301 to A*3317 alleles								
Well ⁵								
9	10	11	12	13	14	15	16	
160	215	165	235	95	115	140	245	Length of spec.
185	285							PCR product(s)
1070	1070	1070	1070	1070	1070	1070	1070	Length of int.
								pos. control ¹
97	97	448	97	395	652	482	97	5'-primer ²
5'-TCA ^{3'}	5'-TCA ^{3'}	5'-CCT ^{3'}	5'-TCA ^{3'}	5'-gCC ^{3'}	5'-CTg ^{3'}	5'-ggC ^{3'}	5'-TCA ^{3'}	
218	270	570	290	448	727	583	299	3'-primer(s) ³
5'-gCC ^{3'}	5'-ACT ^{3'}	5'-CCg ^{3'}	5'-CAg ^{3'}	5'-CAA ^{3'}	5'-CCA ^{3'}	5'-gTg ^{3'}	5'-CCg ^{3'}	
240	341							
5'-ggA ^{3'}	5'-CgT ^{3'}							
9	10	11	12	13	14	15	16	Well No.
								HLA-A allele ⁴
								*3301
								*330301-330302
								*3304
								*3305
								*3306
								*3307
9	10							*3308
9								*3309
		11						*3310
			12					*3311
				13				*3312
	10							*3313
9								*3314
					14			*3315
						15		*3316
							16	*3317
								*0241, 0265, 0280, 9217, 9235, 2462, 250101-2506, 260101- 2635, 310102-310203, 3109- 3122, 320101-3203, 3205-3212, 3214-3216, 340101-3340102, 3405, 3406, 4301, 6601-6603, 6605-6607, 7401-7412N
					14			*9240
9	10	11	12	13	14	15	16	Well No.

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Length of spec.	205	205	155	210	90	175	105	125
PCR product(s)								
Well No.	1	2	3	4	5	6	7	8
*230301, 2903								
*2482, 3103								
*3102, 3107, 3108				4				
*3105, 3213				4				
*6604		2	3					
*6829	1							
HLA-A allele 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-A*33 subtyping. .

In addition, wells number 2 and 5 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, 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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160	215	165	235	95	115	140	245	Length of spec. PCR product(s)
185	285							Well No.
9	10	11	12	13	14	15	16	
		11						*230301, 2903
	10							*2482, 3103
	10							*3102, 3107, 3108
		11						*3105, 3213
								*6604
			12					*6829
								HLA-A allele ⁴
9	10	11	12	13	14	15	16	Well No.

⁴The sequence of the A*3302 allele has been shown to be identical to A*3303.

⁵Primer mix 9: Specific PCR fragment of 160 bp in the A*3314 allele. Specific PCR fragment of 185 bp in the A*3308 and 3309 alleles.

Primer mix 10: Specific PCR fragment of 215 bp in the A*3308 and A*2482, 3102, 3107 and 3108 alleles. Specific PCR fragment of 285 bp in the A*3313 and A*3103 alleles.

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CELL LINE VALIDATION SHEET																				
HLA-A*33 SSP subtyping kit																				
				Lot No.:	Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
					200509301	200509302	200509303	200620804	200734905	200848306	200509307	200509308	200848309	200848310	200734911	200848312	200848313	200848314	200848315	200848316
	IHWC cell line	A*	A*																	
1	9001 SA	*2402			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707	*0201			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324	*0101			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373	*3001			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*0101			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM	*0201	*2603		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL	*2601			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
8	9007 DEM	*0201			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*2601			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3	*2402			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT	*2902			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*0201			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9067 BTB	*0201			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*3101			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*2402			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*2902			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9008 WILJON	*2501			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*3303	*7401		+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16	*0201			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*0201			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*0217			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*0201			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*0201	*3401		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*3201			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*3301			+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*0205			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*2410	*2901		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB	*0201	*2902		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*2902			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*3001	*6802		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*3002			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*0201			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*3101			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*3101			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*3201			+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*0206	*1101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB	*0301			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*0217			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML	*0101	*0301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*0207	*3001		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*0301			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*0207			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*0207			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*6601			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*2301	*2402		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU	*0301			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*0216	*0301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND	*0201	*1101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-A*33 SSP

Product number: 101.432-12u – without *Taq* polymerase
Lot number: 80E
Expiry date: 2010-June-01
Number of tests: 12
Number of wells per test: 16

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2005-076-01	9	2008-483-09
2	2005-076-02	10	2008-483-10
3	2005-076-03	11	2007-349-11
4	2005-076-04	12	2008-483-12
5	2007-349-05	13	2008-483-13
6	2008 483-06	14	2008-483-14
7	2005-076-07	15	2008-483-15
8	2005-076-08	16	2008-483-16

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 5 to 11 were available. The specificities of the primers in primer solutions 5, 9, 10 and 11 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 6 and 8 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solution 7 it was only possible to test the 3'-primer, the 5'-primer was not possible to test.

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

Date of approval: 2009-June-10

Approved by:

Quality Control, Supervisor

Lot No.: **80E**

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Declaration of Conformity

Product name: *Olerup* SSP® HLA-A*33
Product number: 101.432-12u
Lot number: 80E

Intended use: HLA-A*33 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 II List B, conformity assessed using Annex IV, 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.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom. (Notified Body number: 0088.)

Saltsjöbaden, Sweden
2009-June-10

Olle Olerup

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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**.