

Olerup SSP[®] DRB1*16

Product number:	101.126-12 – including <i>Taq</i> polymerase
Lot number:	04K
Expiry date:	2012-May-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. 04K.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] DRB1*16 LOT

The DRB1*16 specificity and interpretation tables have been updated for the DRB1 alleles described since the previous *Olerup SSP*[®] DRB1*16 lot was made (Lot No. 63F).

Three wells have been added to the DRB1*16 kit,
wells **14 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
3	-	Modified	Modified 3'primers for improved specificity.
14	New	New	New primer pair for the DRB1*16:14 allele.
15	New	New	New primer pair for the DRB1*16:15 allele.
16	New	New	New primer pair for the DRB1*16:16 allele.

PRODUCT DESCRIPTION

DRB1*16 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*16:01 to DRB1*16:16 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	16

The 16 well cut PCR plate is marked with 'DRB1*16' in silver/gray ink.

Well No. 1 is marked with the Lot No. '04K'.

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 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 DRB1*16 PCR-SSP subtypings will be influenced by the DRB1*01:23, DRB1*04:53, four DRB1*11, most DRB1*12, two DRB1*13 alleles and the DRB1*15, when present on the other haplotype. In addition, primer mix 16 will amplify the DRB5*01:13 allele.

UNIQUELY IDENTIFIED ALLELES

All the DRB1*16 alleles, i.e. **DRB1*16:01 to DRB1*16:16**, recognized by the HLA Nomenclature Committee in April 2010¹ will give rise to unique amplification patterns by the primers in the DRB1*16 subtyping kit.

The DRB1*16 subtyping kit cannot distinguish the DRB1*16:01:01 and DRB1*16:01:02 alleles, the DRB1*16:02:01 and DRB1*16:02:02 alleles or the DRB1*16:05:01 and DRB1*16:05:02 alleles.

¹DRB1 alleles listed on the IMGT/HLA web page 2010-April-01, release 3.0.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 15 DRB1*16 alleles can be combined in 120 homozygous and heterozygous combinations. Twenty-three of these genotypes do not give rise to unique amplification patterns.

+++-----	++-----	16:01,16:10 = 16:02,16:09 = 16:09,16:10
++-----	++-----	16:02,16:10 = 16:10,16:10
++-----	--+-----	16:02,16:11 = 16:11,16:11
++-----	-----+--	16:02,16:14 = 16:14,16:14
++-----	-----++	16:02,16:16 = 16:16,16:16
+--+-----	-----	16:01,16:03 = 16:03,16:03
+--+-----	-----	16:01,16:04 = 16:04,16:04
+--+-----	-----	16:01,16:08 = 16:08,16:08
+--+-----	++-----	16:01,16:09 = 16:09,16:09
+--+-----	-----+---	16:01,16:13N = 16:13N,16:13N
+-----+-	-----	16:05,16:07 = 16:07,16:07

16:01 = 16:01:01 and 16:01:02
16:02 = 16:02:01 and 16:02:02
16:05 = 16:05:01 and 16:05:02

SPECIFICITY TABLE

DRB1*16 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for DRB1*16 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB1*16 alleles ³	Other amplified DRB1 alleles ⁴
1	260 bp	515 bp	*16:01:01- 16:05:02, 16:07- 16:11, 16:13N- 16:14, 16:16	*15:02:01-15:02:07, 15:08, 15:11, 15:14-15:15, 15:19, 15:26-15:27, 15:29-15:31, 15:34, 15:38-15:39, 15:44
2	200 bp	515 bp	*16:02:01- 16:02:02, 16:10- 16:11, 16:14, 16:16	
3	200 bp	430 bp	*16:01:01- 16:01:02, 16:03- 16:04, 16:08- 16:09, 16:13N, 16:15	
4	215 bp	430 bp	*16:03	
5	220 bp	430 bp	*16:04	*15:21
6	200 bp	430 bp	*16:05:01- 16:05:02, 16:07	*15:10, 15:21
7	160 bp	515 bp	*16:07	
8⁵	110 bp	430 bp	*16:08	
9	140 bp	430 bp	*16:09-16:10	*15:01:01:01-15:06, 15:08, 15:10, 15:12-15:27, 15:29- 15:33, 15:35-15:44
10⁵	115 bp	430 bp	*16:09-16:10	*11:01:03, 11:01:10- 11:01:11, 11:19:02, 12:01:01, 12:01:03- 12:02:03, 12:03:02-12:10, 12:12-12:15, 12:17-12:20, 13:02:02, 13:77
11	215 bp	430 bp	*16:11	
12	220 bp	515 bp	*16:12	
13	155 bp	430 bp	*16:13N	
14	175 bp	430 bp	*16:14	
15⁵	80 bp	430 bp	*16:15	*01:23, 04:53, 12:01:01, 12:01:03-12:02:03, 12:03:02-12:06, 12:08- 12:15, 12:17-12:21, 13:77

16⁵	85 bp	430 bp	*16:16	*11:01:03, 11:01:10- 11:01:11, 11:19:02, 12:04, DRB5*01:13
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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 DRB*16 SSP subtypings.

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 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB1*16 subtyping.

In addition, wells number 2, 7 and 12 contain the primer pair giving rise to the longer, 515 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.

³For several DRB alleles only partial second exon nucleotide sequences are available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. We assume that unknown sequences in the first hyperpolymorphic region of the second exon of DRB alleles are conserved within allelic groups and that unknown sequences of codons 87 to 92 are identical with the DRB1*01:01 consensus sequence.

⁴Due to the sharing of sequence motifs between DRB1 alleles, primer mixes 1, 5, 6, 9, 10, 15 and 16 will amplify other DRB1 alleles. In addition, primer mix 16 will amplify the DRB5*01:13 allele.

⁵Specific PCR fragments shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR bands.

INTERPRETATION TABLE								
DRB1*16 SSP subtyping								
Amplification patterns of the DRB1*1601 to 1616 alleles								
	Well							
	1	2	3	4	5	6	7	8
Length of spec.	260	200	200	215	220	200	160	110
PCR product								
Length of int.	515	515	430	430	430	430	515	430
pos. control ¹								
5'-primer ²	13 (126) 5' -A gg 3'	13 (126) 5' -A gg 3'	13 (126) 5' -A gg 3'	13 (126) 5' -A gg 3'	13 (126) 5' -A gg 3'	13 (126) 5' -A gg 3'	27 (167) 5' -CCC 3'	13 (126) 5' -A gg 3'
3'-primer(s) ³	86 (344) 5' -CAC 3'	67 (286) 5' -gAg 3'	67 (286) 5' -gAA 3'	72 (301) 5' -ggC 3'	74 (307) 5' -CAg 3'	67 (286) 5' -gAT 3'	67 (286) 5' -gAT 3'	37 (197) 5' -CgT 3'
			67 (286) 5' -gAA 3'			67 (286) 5' -gAT 3'	67 (286) 5' -gAT 3'	
Well No.	1	2	3	4	5	6	7	8
DRB1 allele ⁴								
*16:01:01-16:01:02	1		3					
*16:02:01-16:02:02	1	2						
*16:03	1		3	4				
*16:04	1		3		5			
*16:05:01-16:05:02	1					6		
*16:07	1					6	7	
*16:08	1		3					8
*16:09	1		3					
*16:10	1	2						
*16:11	1	2						
*16:12								
*16:13N	1		3					
*16:14	1	2						
*16:15			3					
*16:16	1	2						
*01:23, 04:53, 12:11, 12:21								
*11:01:03, 11:01:10-11:01:11, 11:19:02								
*12:01:01, 12:01:03-12:02:03, 12:03:02, 12:05-12:06, 12:08- 12:10, 12:12-12:15, 12:17-12:20, 13:77								
*12:04								
Well No.	1	2	3	4	5	6	7	8

INTERPRETATION TABLE

DRB1*16 SSP subtyping

Amplification patterns of the DRB1*1601 to 1616 alleles

Well								
9	10	11	12	13	14	15	16	
140	115	215	220	155	175	80	85	Length of spec. PCR product
430	430	430	515	430	430	430	430	Length of int. pos. control ¹
13 (126)	47 (227)	14 (127)	13 (126)	13 (126)	13 (126)	72 (303)	58 (261)	5'-primer ²
5' -Agg 3'	5' -gTT 3'	5' -ggA 3'	5' -AAg 3'	5' -Agg 3'	5' -Agg 3'	5' -CgC 3'	5' -gAg 3'	
47 (227)	72 (303)	72 (303)	72 (303)	52 (241)	57 (258)	86 (344)	72 (303)	3'-primer(s) ³
5' -ggA 3'	5' -gCg 3'	5' -gCg 3'	5' -gCg 3'	5' -CTA 3'	5' -gCT 3'	5' -CCA 3'	5' -gCg 3'	
9	10	11	12	13				Well No.
								DRB1 allele ⁴
								*16:01:01-16:01:02
								*16:02:01-16:02:02
								*16:03
								*16:04
								*16:05:01-16:05:02
								*16:07
								*16:08
9	10							*16:09
9	10							*16:10
		11						*16:11
			12					*16:12
				13				*16:13N
					14			*16:14
						15		*16:15
							16	*16:16
						15		*01:23, 04:53, 12:11, 12:21
	10						16	*11:01:03, 11:01:10-11:01:11, 11:19:02
	10					15		*12:01:01, 12:01:03-12:02:03, 12:03:02, 12:05-12:06, 12:08- 12:10, 12:12-12:15, 12:17-12:20, 13:77
	10					15	16	*12:04
9	10	11	12	13	14	15	16	Well No.

Lot No.: **04K**

Lot-specific information

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Length of spec.	260	200	200	215	220	200	160	110
PCR product								
Well No.	1	2	3	4	5	6	7	8
*12:07, 13:02:02								
*15:01:01:01-15:01:12, 15:03:01:01-15:06, 15:12-15:13, 15:16-15:18, 15:20, 15:22-15:25, 15:32-15:33, 15:35-15:37, 15:40- 15:43								
*15:02:01-15:02:07, 15:08, 15:14- 15:15, 15:19, 15:26-15:27, 15:29- 15:31, 15:38-15:39, 15:44	1							
*15:10						6		
*15:11, 15:34	1							
*15:21					5	6		
DRB5*01:13								
DRB1 allele ⁴								
Well No.	1	2	3	4	5	6	7	8

140	115	215	220	155	175	80	85	Length of spec. PCR product
9	10	11	12	13	14	15	16	Well No.
	10							*12:07, 13:02:02
9								*15:01:01-01-15:01:12, 15:03:01-01-15:06, 15:12-15:13, 15:16-15:18, 15:20, 15:22-15:25, 15:32-15:33, 15:35-15:37, 15:40- 15:43
9								*15:02:01-15:02:07, 15:08, 15:14- 15:15, 15:19, 15:26-15:27, 15:29- 15:31, 15:38-15:39, 15:44
9								*15:10
								*15:11, 15:34
9								*15:21
							16	DRB5*01:13
9	10	11	12	13	14	15	16	DRB1 allele⁴ Well No.

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 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB1*16 subtyping.

In addition, wells number 2, 7 and 12 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

²The codon, and in parenthesis the nucleotide, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given. Codon and 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 codon, and in parenthesis the nucleotide, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given. Codon and 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 DRB1*16:06 allele has been shown to be identical to DRB1*16:05:01.

CELL LINE VALIDATION SHEET																				
DRB1*16 SSP subtyping kit																				
				Well																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Prod. No.:	200628601	200628602	201072103	200628604	200628605	200628606	200628607	200628608	200628609	200628610	200843411	200843412	200843413	201072114	201072115	201072116
IHC cell line		DRB1																		
1	9001 SA	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707	*15:02	*04:05	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
3	9011 E4181324	*15:02		+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
4	9275 GU373	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*16:01		+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM	*04:07	*08:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025 DEJ	*04:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*04:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3	*04:05		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*08:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*09:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540	*03:01	*13:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*09:01	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16	*12:01		-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
20	9059 SLE005	*13:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*14:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*13:02	*14:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*08:03	*14:14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*04:05	*10:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB	*04:16	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*03:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*13:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*04:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*04:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*04:03	*04:06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB	*13:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*14:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML	*03:01	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*07:01	*09:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*13:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*08:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*09:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*14:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU	*15:01		-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
47	9045 TUBO	*11:04	*12:01	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
48	9303 TER-ND	*01:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® DRB1*16 SSP

Product number: 101.126-12 – including *Taq* polymerase
Lot number: 04K
Expiry date: 2012-May-01
Number of tests: 12
Number of wells per test: 16

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2006-286-01	9	2006-286-09
2	2006-286-02	10	2006-286-10
3	2010-721-03	11	2008-434-11
4	2006-286-04	12	2008-434-12
5	2006-286-05	13	2008-434-13
6	2006-286-06	14	2010-721-14
7	2006-286-07	15	2010-721-15
8	2006-286-08	16	2010-721-16

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 4, 6 to 8, 11 to 14 and 16 were available. The specificities of the primers in primer solutions 6, 8 and 16 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 4, 13 and 14 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solution 7, 11 and 12 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solution 3, one 3'-primer was not possible to test.

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

Date of approval: 2010-May-28

Approved by:

Quality Control, Supervisor

Lot No.: **04K**

Lot-specific information

www.olerup-ssp.com

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*16
Product number: 101.126-12
Lot number: 04K

Intended use: DRB1*16 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 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
2010-May-28

Olle Olerup
Managing Director

Lot No.: **04K**

Lot-specific information

www.olerup-ssp.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-ssp.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**.