Visit www.olerup-ssp.com for "Instructions for Use" (IFU)

Lot No.: 64R Lot-specific information

Olerup SSP® HLA-A*34

Product number: 101.425-06 – including *Taq* pol.

101.425-06u – without *Taq* pol.

Lot number: 64R

Expiry date: 2015-May-01

Number of tests: 6 Number of wells per test: 8

Storage - pre-aliquoted primers: dark at -20°C

PCR Master Mix: -20°C
 Adhesive PCR seals
 Product Insert
 RT

This Product Description is only valid for Lot No. 64R.

Changes compared to the previous *OLERUP* SSP® HLA-A*34 Lot (73M)

The HLA-A*34 kit is updated for new alleles to enable separation of:

- Confirmed¹ alleles as listed in the IMGT/HLA database
- Polymorphisms in exons outside of the region encoding the peptide binding domain
- Null and Alternatively expressed alleles

The Lot-specific information for HLA-A*34 including and without *Taq* polymerase is now described in one common Product Insert.

The HLA-A*34 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup* SSP® HLA-A*34 lot was made **(Lot No. 73M)**.

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	Added	-	5'-primer added for the A*34:10N allele

¹As described in section Uniquely Identified Alleles.

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Lot No.: 64R Lot-specific information

PRODUCT DESCRIPTION

HLA-A*34 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*34:01 to A*34:10N alleles.

PLATE LAYOUT

Each test consists of 8 PCR reactions in an 8 well cut PCR plate.

4	2	2	4	E	c	7	0
_ I		3	4) j	O	<i>'</i>	O

The 8 well cut PCR plate is marked with 'A34' in silver/gray ink.

Well No. 1 is marked with the Lot No. '64R'.

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 heat-sealed with a PCR-compatible foil.

Please note: When removing each 8 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-A*34 SSP subtypings will be influenced by three A*01, five A*02, five A*03, most A*11, four A*25, twelve A*26, three A*31, the A*32:15, most A*66 and the A*68:71 alleles when present on the other haplotype. In addition, the B*15:82, B*15:260 and B*40:186 alleles will be amplified by primer mix 5 and the C*07:81 and C*07:243 alleles will be amplified by primer mix 6.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*34 alleles, i.e. **A*34:01 to A*34:10N alleles**, recognized by the HLA Nomenclature Committee in October 2012¹ will be amplified by the primers in the HLA-A*34 subtyping kit.

The HLA-A*34 kit enables separation of the confirmed HLA-A*34 alleles as listed in the IMGT/HLA database. An HLA allele is listed as confirmed by IMGT/HLA if it has been sequenced by more than a single laboratory or from multiple sources. Current allele confirmation status for HLA-A*34 alleles is listed below.

The HLA-A*34 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.

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Lot No.: 64R Lot-specific information

The HLA-A*34 subtyping kit cannot distinguish the silent mutations in the A*34:01:01-34:01:02 or the *34:02:01-34:02:03 alleles.

¹HLA-A alleles listed on the IMGT/HLA web page 2012-October-17, release 3.10.0, www.ebi.ac.uk/imgt/hla.

ALLELE CONFIRMATION STATUS

Allele Status ¹		Allele	Status ¹
A*34:01:01	Confirmed	A*34:08	Confirmed
A*34:01:02	Unconfirmed	A*34:09	Unconfirmed
A*34:02:01	Confirmed	A*34:10N	Unconfirmed
A*34:02:02	Unconfirmed		
A*34:02:03	Unconfirmed		
A*34:03	Confirmed		
A*34:04	Unconfirmed		
A*34:05	Confirmed		
A*34:06	Unconfirmed		
A*34:07	Unconfirmed		

¹Allele status "confirmed" or "unconfirmed" as listed on the IMGT/HLA web page 2012-October-17, release 3.10.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 13 alleles generate 10 amplification patterns that can be combined in 55 homozygous and heterozygous combinations. 30 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products were not considered in these calculations.

```
+--+--
                *34:03, *34:06 = *34:06, *34:06
+-+---+
                *34:02:01, *34:08 = *34:02:01, *34:09 = *34:08, *34:08 = *34:08,
                *34:09
+-+--+--
                *34:02:01, *34:10N = *34:10N, *34:10N
+-+-+---
                *34:02:01, *34:04 = *34:04, *34:04
++---+--
                *34:01:01, *34:05 = *34:05, *34:05
+-+--+
                *34:08, *34:10N = *34:09, *34:10N
+-+-+-+
                *34:04, *34:08 = *34:04, *34:09
                *34:03, *34:08 = *34:03, *34:09
                *34:02:01, *34:06 = *34:03, *34:07 = *34:06, *34:07
+-++--+-
                *34:01:01, *34:08 = *34:01:01, *34:09
+++--+--
                *34:01:01, *34:10N = *34:02:01, *34:05 = *34:05, *34:10N
+-++--++
                *34:06, *34:08 = *34:06, *34:09
                *34:05, *34:08 = *34:05, *34:09
+++--+
```

^{*34:01:01 = *34:01:01-34:01:02}

^{*34:02:01 = *34:02:01-34:02:03}

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Lot No.: 64R

Lot-specific information

SPECIFICITY TABLE

HLA-A*34 SSP subtyping

Specificities and sizes of the PCR products of the 8 primer mixes used for HLA-A*34 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA- A*34 alleles	Other amplified HLA Class I alleles ³
14	100 bp	800 bp	*34:01:01-34:06, 34:08, 34:10N	*01:13, 01:17, 03:63, 03:88, 11:01:01-11:11, 11:13-11:16, 11:20-11:27, 11:29-11:39, 11:41-11:52Q, 11:54-11:95, 11:97, 11:99N-11:105, 11:107-11:120, 11:122-11:132, 25:02, 26:13, 26:19, 26:33, 66:01, 66:04, 66:06-66:11, 66:13-66:14, 66:17-66:19
2 ⁴	110 bp	1070 bp	*34:01:01- 34:01:02, 34:05	*26:48, 26:69
3	195 bp	1070 bp	*34:02:01- 34:02:03, 34:04, 34:07-34:10N	
4 ⁵	135 bp	800 bp	*34:03, 34:06	*03:01:19, 25:09, 26:14, 26:18, 26:28, 26:73, 31:03-31:04
5	200 bp	800 bp	*34:04	*31:01:07, B*15:82, B*15:260, B*40:186
6 ^{6,7}	155 bp, 185 bp	1070 bp	*34:05, 34:10N	*02:91, 02:322, 03:94, C*07:81, C*07:243
7 ⁸	140 bp, 215 bp	1070 bp	*34:06-34:07	*11:96, 26:18, 31:03- 31:04
8 ⁹	200 bp, 360 bp	800 bp	*34:08-34:09	*01:51, 02:55, 02:135, 02:309, 03:01:19, 03:24, 25:03, 25:13, 26:20, 26:30, 26:65, 31:04, 32:15, 66:02-66:03, 66:12, 66:16, 68:71

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Lot No.: 64R Lot-specific information

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-A*34 SSP typings.

When the primers in a primer mix can give rise to HLA-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits the respective lengths of the HLA-specific PCR product(s) are given for the alleles amplified by these primer mixes.

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 inherit 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 addition, wells number 4, 5 and 8 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-A alleles non-HLA-A*34 alleles will be amplified by primer mixes 1, 2 and 4 to 8. In addition, the B*15:82, B*15:260 and B*40:186 alleles will be amplified by primer mix 5 and the C*07:81 and C*07:243 alleles will be amplified by primer mix 6.

⁴HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁵Primer mix 4 may give rise to a lower yield of HLA-specific PCR product than the other A*34 primer mixes.

primer mixes. ⁶Primer mix 6 may have tendencies of unspecific amplifications, and may also have a tendency to giving rise to primer oligomer formation.

⁷Primer mix 6: Specific PCR fragment of 155 bp in the A*34:05 and the A*02:91, 02:322 and 03:94 and in theC*07:81 and C*07:243 alleles. Specific PCR fragment of 185 bp in the A*34:10N allele.

⁸Primer mix 7: Specific PCR fragment of 140 bp in the A*34:06 and the A*26:18 and 31:03-31:04 alleles. Specific PCR fragment of 215 bp in the A*34:07 and the A*11:96 alleles. ⁹Primer mix 8: Specific PCR fragment of 200 bp in the A*34:08 and the A*01:51, 02:55, 03:24,

⁹Primer mix 8: Specific PCR fragment of 200 bp in the A*34:08 and the A*01:51, 02:55, 03:24, 25:03, 26:20, 32:15 and 68:71 alleles. Specific PCR fragment of 360 bp in the A*34:09 and the A*02:135, 02:309, 03:01:19, 25:13, 26:30, 26:65, 31:04, 66:02-66:03, 66:12 and 66:16 alleles.

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Lot No.: 64R Lot-specific information

INTERPRETATION TABLE								
HLA-A*34 SSP subtyping								
Amplification patterns of the HLA-A*34:01 to 34:10N alleles								
,pear passer	<u> </u>				ell ⁵			
	1	2	3	4	5	6	7	8
Length of spec.	100	110	195	135	200	155	140	200
PCR product						185	215	360
Length of int.	800	1070	1070	800	800	1070	1070	800
pos. control ¹								
5'-primer(s) ²	282	270	363	423	78	415	103	102
, p	^{5'} -CAg ^{3'}	^{5'} -AAA ^{3'}	^{5'} -ATA ^{3'}	^{5'} -gCT ^{3'}	^{5'} -TCC ³	^{5'} -ggT ^{3'}	^{5'} -CCT ^{3'}	^{5'} -ACA ^{3'}
						445	423	341
						^{5'} -TCT ^{3'}	^{5'} -gCT ^{3'}	^{5'} -ggC ^{3'}
3'-primer(s) ³	341	341	517	517	238	559	277	259
briller(s)						^{'5'} -CgT ^{3'}		
							524	418
								^{5'} -gTC ^{3'}
Well No.	1	2	3	4	5	6	7	8
HLA-A allele ⁴								
*34:01:01- 34:01:02	1	2						
*34:02:01- 34:02:03	1		3					
*34:03	1			4				
*34:04	1		3		5			
*34:05	1	2				6		
*34:06	1			4			7	
*34:07			3				7	
*34:08	1		3					8
*34:09			3					8
*34:10N	1		3			6		
*01:13, 01:17, 03:63, 03:88,								
11:01:01-11:11, 11:13-11:16,								
11:20-11:27, 11:29-11:39, 11:41-								
11:52Q, 11:54-11:95, 11:97,	1							
11:99N-11:105, 11:107-11:120,	'							
11:122-11:132, 25:02, 26:13,								
26:19, 26:33, 66:01, 66:04, 66:06-								
66:11, 66:13-66:14, 66:17-66:19								
Well No.	1	2	3	4	5	6	7	8

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Lot No.: 64R Lot-specific information

Length of spec.	100	110	195	135	200	155	140	200
PCR product						185	215	360
Well No.	1	2	3	4	5	6	7	8
*01:51, 02:55, 02:135, 02:309, 03:24, 25:03, 25:13, 26:20, 26:30, 26:65, 32:15, 66:02-66:03, 66:12, 66:16, 68:71								8
*02:91, 02:322, 03:94, <i>C*07:81, C*07:243</i>						6		
*03:01:19				4				8
*11:96							7	
*25:09, 26:14, 26:28, 26:73				4				
*26:18, 31:03				4			7	
*26:48, 26:69		2						
*31:01:07, <i>B*15:82, B*15:260, B*40:186</i>					5			
*31:04				4			7	8
HLA-A allele								
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 addition, wells number 4, 5 and 8 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

bp, internal positive control band in order to allow kit identification.

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

of the 3 terminal nucleotides of the primer is given.

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

⁴HLA-A*34 alleles in bold lettering are listed as confirmed alleles on the on the IMGT/HLA web page www.ebi.ac.uk/imgt/hla, release 3.10.0, October 2012.

⁵Primer mix 6: Specific PCR fragment of 155 bp in the A*34:05 and the A*02:91, 02:322 and 03:94 and in the C*07:81 and C*07:243 alleles. Specific PCR fragment of 185 bp in the A*34:10N allele.

Primer mix 7: Specific PCR fragment of 140 bp in the A*34:06 and the A*26:18 and 31:03-31:04 alleles. Specific PCR fragment of 215 bp in the A*34:07 and the A*11:96 alleles.

Primer mix 8: Specific PCR fragment of 200 bp in the A*34:08 and the A*01:51, 02:55, 03:24, 25:03, 26:20, 32:15 and 68:71 alleles. Specific PCR fragment of 360 bp in the A*34:09 and the A*02:135, 02:309, 03:01:19, 25:13, 26:30, 26:65, 31:04, 66:02-66:03, 66:12 and 66:16 alleles.

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Lot No.: 64R Lot-specific information

LOT	Lot No.: 04K Lot-specific information											
	CELL LINE VALIDATION SHEET HLA-A*34 SSP subtyping kit											
		IILA-A	37 001	Subty	/ P !!	<u> </u>	KI		<u> </u>			
					<u> </u>	_	_		ell	_	_	
					1	2	3	4	5	6	7	8
				Lot No.:	201073901	201073902	201073903	201073904	201073905	201211006	201073907	201191008
	IHV	VC cell line	A*	A*								
1	9001		*24:02		-	-	-	-	-	-	-	-
2	9280	LK707	*02:01		-	-	-	-	-	-	-	-
3	9011	E4181324	*01:01		-	-	-	-	-	-	-	-
4	9275	GU373	*30:01		-	-	-	-	-	-	-	-
5	9009	KAS011	*01:01		-	-	-	-	-	-	-	-
6	9353	SM	*02:01	*26:03	-	-	-	-	-	-	-	-
7	9020		*26:01		-	-	-	-	-	-	-	-
8	9025		*31:01		-	-	-	-	-	-	-	-
9	9026	YAR	*26:01		-	-	-	-	-	-	-	-
10	9107	LKT3	*24:02		-	-	-	-	-	-	-	-
11	9051	PITOUT	*29:02		-	-	-	-	-	-	-	-
12	9052	DBB	*02:01		-	-	-	-	-	-	-	-
13	9004	JESTHOM	*02:01		-	-	-	-	-	-	-	-
14	9071	OLGA	*31:01		-	-	-	-	-	-	-	-
15	9075	DKB	*24:02		-	-	-	-	-	-	-	-
16	9037	SWEIG007	*29:02		-	-	-	-	-	-	-	-
17	9282	CTM3953540	*03:01	*80:01	-	-	-	-	-	-	-	-
18	9257	32367	*33:03	*74:01	-	-	-	-	-	-	-	-
19	9038	BM16	*02:01		-	-	-	-	-	-	-	-
20	9059	SLE005	*02:01		-	-	-	-	-	-	-	-
21		AMALA	*02:17		-	-	-	-	-	-	-	-
22		KOSE	*02:01		-	-	-	-	-	-	-	-
23	9124		*02:01	*34:01	+	+	-	-	-	-	-	-
24	9035	JBUSH	*32:01		-	-	-	-	-	-	-	-
25		IBW9	*33:01		-	-	-	-	-	-	-	-
26		WT49	*02:05		-	-	-	-	-	-	-	-
27		CH1007	*24:10	*29:01	-	-	-	-	-	-	-	-
28		BEL5GB	*02:01	*29:02	-	-	-	-	-	-	-	-
29	9050		*29:02		-	-	-	-	-	-	-	-
30	9021		*30:01	*68:02	-	-	-	-	-	-	-	-
31		DUCAF	*30:02		-	-	-	-	-	-	-	-
32	9297		*02:01		-	-	-	-	-	-	-	-
33		MT14B	*31:01		-	-	-	-	-	-	-	-
34	9104		*31:01		-	-	-	-	-	-	-	-
35		SSTO	*32:01		-	-	-	-	-	-	-	-
36		KT17	*02:06	*11:01	+	-	-	-	-	-	-	-
37		HHKB	*03:01		-	-	-	-	-	-	-	-
38	9099		*02:17	405.5	-	-	-	-	-	-	-	-
39	9315		*01:01	*03:01	-	-	-	-	-	-	-	-
40		WHONP199	*02:07	*30:01	_	_	-	-	-	-	_	_
41		H0301	*03:01		-	-	-	-	-	-	-	-
42		TAB089	*02:07		-	-	-	-	-	-	-	-
43		T7526	*02:06	*02:07	-	-	-	-	-	-	-	-
44	9057		*66:01	1.2	+	-	-	-	-	-	-	-
45		SHJO	*23:01	*24:02	-	-	-	-	-	-	-	-
46		SCHU	*03:01		-	-	-	-	-	-	-	-
47		TUBO	*02:16	*03:01	-	-	-	-	-	-	-	-
48	9303	TER-ND	*02:01	*11:01	+	-	-	-	-	-	-	-

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Lot No.: 64R Lot-specific information

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-A*34 SSP

Product number: 101.425-06 – including *Taq* pol.

101.425-06u – without *Taq* pol.

Lot number: 64R

Expiry date: 2015-May-01

Number of tests: 6 Number of wells per test: 8

Well specifications:

Well No.	Production No.
1	2010-739-01
2	2010-739-02
3	2010-739-03
4	2010-739-04
5	2010-739-05
6	2012-110-06
7	2010-739-07
8	2011-910-08

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

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

Date of approval: 2012-December-19

Approved by:

Production Quality Control

Visit <u>www.olerup-ssp.com</u> for "Instructions for Use" (IFU)

Lot No.: 64R Lot-specific information

Declaration of Conformity

Product name: Olerup SSPTM HLA-A*34

Product number: 101.425-06/06u

Lot number: 64R

Intended use: HLA-A*34 high resolution histocompatibility testing

Manufacturer: Olerup SSP AB

Franzengatan 5

SE-112 51 Stockholm, 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, Franzengatan 5, SE-112 51 Stockholm, Sweden.

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

Stockholm, Sweden 2012-December-19

Ann-Cathrin Jareman Head of QA and Regulatory Affairs

 $\label{thm:continuity} \begin{tabular}{ll} Visit $\underline{www.olerup\text{-}ssp.com}$ for \\ \begin{tabular}{ll} Instructions for Use" (IFU) \end{tabular}$

Lot No.: 64R Lot-specific information

Visit www.olerup-ssp.com for "Instructions for Use" (IFU)

Lot No.: 64R Lot-specific information

ADDRESSES:

Manufacturer:

Olerup SSP AB, Franzengatan 5, SE-112 51 Stockholm, 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.