

Lot No.: **61G**

Lot-specific information

***Olerup* SSP[®] HLA-B*14**

Product number:	101.524-12u – without <i>Taq</i> polymerase
Lot number:	61G
Expiry date:	2011-October-01
Number of tests:	12
Number of wells per test:	11
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. 61G.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP[®] HLA-B*14 Lot

The HLA-B*14 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup* SSP[®] HLA-B*14 lot was made (Lot No. 46E).

Three wells have been added to the HLA-B*14 kit,
wells **9 to 11**.

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

Well	5'-primer	3'-primer	rationale
2	-	Modified	Increased specificity of specific primer pair.
3	-	Modified	Increased specificity of specific primer pair.
9	New	New	New primer pair for the B*1409 allele.
10	New	New	New primer pair for the B*1410 allele.
11	New	New	New primer pair for improved resolution of the B*1408 allele.

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PRODUCT DESCRIPTION

HLA-B*14 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-B*1401 to B*1410 alleles.

PLATE LAYOUT

Each test consists of 11 PCR reactions in a 16 well PCR plate. Wells 12 to 16 are empty.

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

The 16 well cut PCR plate is marked with 'HLA-B*14'.

Well No. 1 is marked with the Lot No. '61G'.

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 HLA-B*14 SSP subtypings will be influenced by most B*07, most B*08, the B*1306, twelve B*15, most B*18, ten B*27, ten B*35, most B*37, the B*38, the B*39, many B*40, the B*41, most B*42, two B*44, the B*4508, three B*46, most B*48, nine B*51, two B*53, most B*54, many B*55, four B*56, the B*5714, three B*58, most B*59, the B*67, the B*7301 and B*81 alleles when present on the other haplotype. In addition, the Cw*0808 allele will be amplified by primer mix 6.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*14 alleles, i.e. **B*1401 to B*1410**, recognized by the HLA Nomenclature Committee in July 2009¹ will give rise to unique amplification patterns by the primers in the HLA-B*14 subtyping kit².

The HLA-B*14 primer set cannot distinguish the B*140201 and B*140202 alleles or the B*140601 and B*140602 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2009-July-17, release 2.26.0, www.ebi.ac.uk/imgt/hla.

²The B*1408, B*3943 and B*3948 alleles will give rise to identical amplification patterns with the HLA-B*14 subtyping kit. The B*1408 allele can be distinguished from the B*3943 and B*3948 alleles by the HLA-B*39 subtyping kit.

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RESOLUTION IN HOMO- AND HETEROZYGOTES

The 10 HLA-B*14 alleles can be combined in 55 homozygous and heterozygous combinations. Fifteen of these genotypes did not give rise to different amplification patterns. The different sizes of the specific PCR products generated by primer mix 5 were not considered in these calculations.

+++--++	--+	1404,1408 = 1406,1407N
+++--++	--+	1401,1404 = 1402,1407N = 1404,1407N
+++--++	--+	1401,1406 = 1402,1408
+++--++	--+	1401,1407N = 1407N,1407N
+++--++	--+	1402,1403 = 1403,1403
+++--++	--+	1402,1404 = 1404,1404
+++--++	++	1402,1409 = 1409,1409

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SPECIFICITY TABLE

HLA-B*14 SSP subtyping

Specificities and sizes of the PCR products of the 11 primer mixes used for HLA-B*14 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*14 alleles	Other amplified HLA Class I alleles ³
1	265 bp	800 bp	*1401-1404, 1407N, 1409	
2⁴	185 bp	1070 bp	*1401, 1407N, 1408, 1410	*380101-3803, 3807-3819, 39010101, 39010102L, 390103-390201, 3903, 3905-390602, 3907 [?] , 3908, 3909, 3911, 3914, 3915, 3918, 391902, 3922, 3924-3944, 3946-3948, 3950, 670101
3⁴	185 bp	800 bp	*140201-1403, 1404 ^w , 1405-140602, 1409	*3526, 3805, 3904, 3907 [?]
4⁵	85 bp	1070 bp	*1403	*1813, 1830, 5129
5⁶	135 bp, 220 bp	1070 bp	*1404, 1407N	*3940N
6⁵	95 bp	1070 bp	*1405	*3819, 3903, 3924, 3937, 4206, 4807, Cw*0808
7	260 bp	1070 bp	*140601, 140602, 1408	*1537, 1538, 180101-180103, 1803-1809, 1812, 1813, 1815, 1817N-1820, 1823N-1826, 1828-1833, 3932, 3943, 3948, 4051, 5106, 5145, 5162
8	250 bp	1070 bp	*1401, 1407N, 1408, 1410	*070201-070207, 070209-0726, 0728, 0730-0735, 0737, 0739-0764, 0766-0769, 0775-0777, 1568, 1571, 180101-1803, 1805-1808, 1810-1815, 1817N-1824, 1826-1833, 1835, 2708, 2712, 2718, 2726, 2733, 2740, 2742, 2744, 3550, 3584, 3705, 3711, 39010101, 39010102L, 390103-3903, 3905-

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				390602, 3907 [?] , 3908-3911, 391301-3920, 3922-3950, 400201-400602, 4008, 4009, 4011, 4018, 4020, 4024, 4026-4029, 4035, 4037, 4039, 4040, 4044, 4050, 4056, 4064, 4068, 4070, 4071, 4074, 4075, 4078, 4082, 4083, 4085, 4086, 4089-4091, 4093- 4095, 4097, 4098, 4201, 4202, 4204-4212, 480101- 4817, 4819, 5402, 5510, 670101-6702, 7301, 8101- 8104N
9	160 bp	1070 bp	*1409	
10	270 bp	1070 bp	*1410	*1822, 3521, 3524, 3581, 4028, 5104, 5146, 5156, 5302, 5306, 5714, 5809
11	135 bp	1070 bp	*1401-1410	*0719, 0731, 0734, 0743, 080101-0810, 081201- 0820, 0822-0824, 0826- 0838, 1306, 1542, 1544, 1550, 1569, 1583, 1586, 1593, 9521, 180101-1809, 1811-1815, 1817N-1820, 1822-1833, 2715, 2728, 3535, 3560, 3587, 3593, 370101-370104, 3703N- 3706, 3708-3714, 380101- 3807, 3809-3819, 39010101, 39010102L, 390103-3920, 3922-3931, 3934-3946, 3948, 3949, 4039, 4051, 4101-4108, 4201, 4202, 4204-4212, 4404, 4456N, 4508, 4603, 4618, 4621, 5121, 5136, 5401, 5402, 5404, 5405N, 5407-5419, 550101-5505, 5507, 5510-5521, 5523, 5525-5535, 5610, 5612, 5618, 5623, 5805, 5820, 5901-5903, 5905, 670101- 6702

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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 HLA-B*14 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 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 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-B*14 subtyping.

In addition, well number 3 contains 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-B alleles some non-HLA-B*14 alleles will be amplified by primer mixes 2 to 8, 10 and 11. In addition, the Cw*0808 allele will be amplified by primer mix 6.

The B*1408, B*3943 and B*3948 alleles will give rise to identical amplification patterns with the HLA-B*14 subtyping kit. The B*1408 allele can be distinguished from the B*3943 and B*3948 alleles by the HLA-B*39 subtyping kit.

⁴Primer mix 2 and 3 might yield less specific PCR product than the other HLA-B*14 primer mixes.

⁵Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁶Primer mix 5: Specific PCR fragment of 135 bp in the B*1407N and B*3940N alleles. Specific PCR fragment of 220 bp in the B*1404 allele.

'w', might be weakly amplified.

'?', nucleotide sequence of the primer matching sequence is not known.

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INTERPRETATION TABLE											
HLA-B*14 SSP subtyping											
Amplification patterns of the B*1401 to B*1410 alleles											
	Well ⁴										
	1	2	3	4	5	6	7	8	9	10	11
Length of spec.	265	185	185	85	135	95	260	250	160	270	135
PCR product					220						
Length of int.	800	1070	800	1070	1070	1070	1070	1070	1070	1070	1070
pos. control ¹											
5'-primer(s) ²	361	103	103	539	91	363	363	103	361	355	560
	5' -AgT 3'	-CCT	-CCg	-gCg	-ggC	-AgC	-Agg	-CCT	-AgT	-TCA	-CAC
					564						
					5' -TgA 3'						
3'-primer(s) ³	583	246	246	583	269	419	583	310	479	583	3 rd I
	5' -gTg 3'	-TAT	-TAT	-gTg	-AgT	-CgA	-gTg	-gTT	-CCC	-gTg	-TAT
		246			3 rd I						
	5' -TAT 3'				5' -TAT 3'						
Well No.	1	2	3	4	5	6	7	8	9	10	11
HLA-B allele											
*1401	1	2						8			11
*140201, 140202	1		3								11
*1403	1		3	4							11
*1404	1		w		5						11
*1405			3			6					11
*140601, 140602			3				7				11
*1407N	1	2			5			8			11
*1408, 3943, 3948 ⁵		2					7	8			11
*1409	1		3						9		11
*1410		2						8		10	11
Well No.	1	2	3	4	5	6	7	8	9	10	11

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Length of spec.	265	185	185	85	135	95	260	250	160	270	135
PCR product					220						
Well No.	1	2	3	4	5	6	7	8	9	10	11
*070201-070207, 070209-071802, 0720-0726, 0728, 0730, 0732, 0733, 0735, 0737, 0739-0742, 0744-0764, 0766-0769, 0775-0777, 1568, 1571, 1810, 1821, 1835, 2708, 2712, 2718, 2726, 2733, 2740, 2742, 2744, 3550, 3584, 400201-400602, 4008, 4009, 4011, 4018, 4020, 4024, 4026, 4027, 4029, 4035, 4037, 4040, 4044, 4050, 4056, 4064, 4068, 4070, 4071, 4074, 4075, 4078, 4082, 4083, 4085, 4086, 4089-4091, 4093-4095, 4097, 4098, 480101-4806, 4808-4817, 4819, 7301, 8101-8104N								8			
*0719, 0731, 0734, 0743, 1802, 1811, 1814, 1827, 3705, 3711, 390202, 3910, 391301, 391302, 3916, 3917, 391901, 3920, 3923, 3945, 3949, 4039, 4201, 4202, 4204-420502, 4207-4212, 5402, 5510, 670102, 6702								8			11
*080101-0810, 081201-0820, 0822-0824, 0826-0838, 1306, 1542, 1544, 1550, 1569, 1583, 1586, 1593, 9521, 2715, 2728, 3535, 3560, 3587, 3593, 370101-370104, 3703N, 3704, 3706, 3708-3710, 3712-3714, 3804, 3806, 3912, 4101-4108, 4404, 4456N, 4508, 4603, 4618, 4621, 5121, 5136, 5401, 5404, 5405N, 5407-5419, 550101-5505, 5507, 5511-5521, 5523, 5525-5535, 5610, 5612, 5618, 5623, 5805, 5820, 5901-5903, 5905											11
*1537, 1538, 5106, 5145, 5162							7				
*180101-180103, 1803, 1805-1808, 1812, 1815, 1817N-1820, 1823N, 1824, 1826, 1828, 1829, 1831-1833							7	8			11
Well No.	1	2	3	4	5	6	7	8	9	10	11

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Length of spec.	265	185	185	85	135	95	260	250	160	270	135
PCR product					220						
Well No.	1	2	3	4	5	6	7	8	9	10	11
*1804, 1809, 1825, 4051							7				11
*1813, 1830				4			7	8			11
*1822								8		10	11
*3521, 3524, 3581, 5104, 5146, 5156, 5302, 5306, 5714, 5809										10	
*3526			3								
*380101-3803, 3807, 3809-3818		2									11
*3805, 3904			3								11
*3808		2									
*3819		2				6					11
*39010101, 39010102L, 390103- 390201, 3905-390602, 3908, 3909, 3911, 3914, 3915, 3918, 391902, 3922, 3925N-3931, 3934-3936, 3938Q, 3939, 3941, 3942, 3944, 3946, 670101		2						8			11
*3903, 3924, 3937		2				6		8			11
*3907		?	?					?			11
*3932		2					7	8			
*3933, 3947, 3950		2						8			
*3940N		2			5			8			11
*4028								8		10	
*4206						6		8			11
*4807						6		8			
*5129				4							
Cw*0808						6					
HLA-B allele											
Well No.	1	2	3	4	5	6	7	8	9	10	11

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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-B*14 subtyping. .

In addition, well number 3 contains 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 or 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.

⁴Primer mix 5: Specific PCR fragment of 135 bp in the B*1407N and B*3940N alleles. Specific PCR fragment of 220 bp in the B*1404 allele.

⁵The B*1408, B*3943 and B*3948 alleles will give rise to identical amplification patterns with the HLA-B*14 subtyping kit. The B*1408 allele can be distinguished from the B*3943 and B*3948 alleles by the HLA-B*39 subtyping kit.

'w', might be weakly amplified.

'?', nucleotide sequence of the primer matching sequence is not known.

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CELL LINE VALIDATION SHEET																
HLA-B*14 SSP subtyping kit																
						Well										
						1	2	3	4	5	6	7	8	9	10	11
					Prod. No.:	200732301	200964502	200964503	200845002	200732305	200732306	200732307	200964502	200964509	200964510	200964511
	IHC cell line		B*													
1	9001	SA	*0702			-	-	-	-	-	-	-	+	-	-	-
2	9280	LK707	*5201	*7301		-	-	-	-	-	-	-	+	-	-	-
3	9011	E4181324	*52011			-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*1510	*5301		-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*3701			-	-	-	-	-	-	-	-	-	-	+
6	9353	SM	*3901	*5101		-	+	-	-	-	-	-	+	-	-	+
7	9020	QBL	*1801			-	-	-	-	-	-	+	+	-	-	+
8	9025	DEU	*3501			-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*3801			-	+	-	-	-	-	-	-	-	-	+
10	9107	LKT3	*5401			-	-	-	-	-	-	-	-	-	-	+
11	9051	PITOUT	*4403			-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*5701			-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*2705			-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*1501	*1520		-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*4001			-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*4002			-	-	-	-	-	-	-	+	-	-	-
17	9282	CTM3953540	*0801	*5501		-	-	-	-	-	-	-	-	-	-	+
18	9257	32367	*1401	*5601		+	+	-	-	-	-	-	+	-	-	+
19	9038	BM16	*1801			-	-	-	-	-	-	+	+	-	-	+
20	9059	SLE005	*4001			-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*1501			-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*3503			-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*4002	*5602		-	-	-	-	-	-	-	+	-	-	-
24	9035	JBUSH	*3801			-	+	-	-	-	-	-	-	-	-	+
25	9049	IBW9	*1402			+	-	+	-	-	-	-	-	-	-	+
26	9285	WT49	*5801			-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*0705	*5101		-	-	-	-	-	-	-	+	-	-	-
28	9320	BEL5GB	*4402	*4403		-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*4403			-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*4201			-	-	-	-	-	-	-	+	-	-	+
31	9019	DUCAF	*1801			-	-	-	-	-	-	+	+	-	-	+
32	9297	HAG	*4102			-	-	-	-	-	-	-	-	-	-	+
33	9098	MT14B	*4001			-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*3801			-	+	-	-	-	-	-	-	-	-	+
35	9302	SSTO	*4402			-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*1501	*3501		-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*0702			-	-	-	-	-	-	-	+	-	-	-
38	9099	LZL	*1501			-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*0801	*2705		-	-	-	-	-	-	-	-	-	-	+
40	9134	WHONP199	*1302	*4601		-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*1402			+	-	+	-	-	-	-	-	-	-	+
42	9066	TAB089	*4601			-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*4601			-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*3801			-	+	-	-	-	-	-	-	-	-	+
45	9239	SHJO	*4201	*5001		-	-	-	-	-	-	-	+	-	-	+
46	9013	SCHU	*0702			-	-	-	-	-	-	-	+	-	-	-
47	9045	TUBO	*5101			-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*3501	*4403		-	-	-	-	-	-	-	-	-	-	-

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CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-B*14 SSP

Product number: 101.524-12u – without Taq polymerase
Lot number: 61G
Expiry date: 2011-October-01
Number of tests: 12
Number of wells per test: 11

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2007-323-01	9	2009-645-09
2	2009-645-02	10	2009-645-10
3	2009-645-03	11	2009-645-11
4	2008-450-04		
5	2007-323-05		
6	2007-323-06		
7	2007-323-07		
8	2009-645-08		

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 solution 4, 5, 6, 9 and 10 were available. The specificities of the primers in primer solution 4, 6 and 10 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 9 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. Additional primers in primer solution 5 were tested by separately adding one additional 5'-primer, respective one additional 3'-primer. In primer solution 5, one 5'-primer and one 3'-primer was not possible to test.

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

Date of approval: 2009-October-23

Approved by:

Quality Control, Supervisor

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Declaration of Conformity

Product name: Olerup SSP[®] HLA-B*14
Product number: 101.524-12u
Lot number: 61G

Intended use: HLA-B*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 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-October-23

Olle Olerup
Managing Director

Lot No.: **61G**

Lot-specific information

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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**.