

## **Olerup SSP<sup>®</sup> HLA-B\*58**

Product number:	101.568-06 – including <i>Taq</i> polymerase
Lot number:	35G
Expiry date:	2011-October-01
Number of tests:	6
Number of wells per test:	21
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. 35G.**

### **CHANGES COMPARED TO THE PREVIOUS OLERUP SSP<sup>®</sup> HLA-B\*58 LOT**

The HLA-B\*58 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP<sup>®</sup>* HLA-B\*58 lot was made (**Lot No. 13F**).

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

Well	5'-primer	3'-primer	rationale
7	Modified	-	Increased yield of specific PCR product.
20	Exchanged	Exchanged	Exchange to new primer pair for the B*5821 allele.

## PRODUCT DESCRIPTION

### HLA-B\*58 SSP subtyping

#### CONTENT

The primer set contains 5'- and 3'-primers for identifying the B\*5801 to B\*5821 alleles.

#### PLATE LAYOUT

Each test consists of 21 PCR reactions in a 24 well PCR plate. Wells 22 to 24 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	empty	empty	empty

The 24 well cut PCR plate is marked with 'HLA-B\*58' in silver/gray ink..

Well No. 1 is marked with the Lot No. '35G'.

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 24 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-B\*58 SSP subtypings will be influenced by many other HLA-B alleles as primer mixes 2, 3, 5, 6, 7, 10, 11, 13, 14 and 19 amplify many non-HLA-B\*58 alleles. In addition, the Cw\*0317 allele will be amplified by primer mix 3, and the A\*2464 and Cw\*0106 alleles will be weakly amplified by primer mix 11.

#### UNIQUELY IDENTIFIED ALLELES

All the HLA-B\*58 alleles, i.e. **B\*5801 to B\*5821** recognized by the HLA Nomenclature Committee in July 2009<sup>1</sup> will give rise to unique amplification patterns by the primers in the HLA-B\*58 subtyping kit.

The HLA-B\*58 primer set cannot distinguish the B\*580101 and B\*580102 alleles.

<sup>1</sup>HLA-A alleles listed on the IMGT/HLA web page 2009-July-17, release 2.26.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

**RESOLUTION IN HOMO- AND HETEROZYGOTES**

The twenty HLA-B\*58 alleles can be combined in 210 homozygous and heterozygous combinations. Eighty-six of these genotypes do not give rise to unique amplification patterns.

The different lengths of the specific PCR products in lane 4 were not considered in these calculations.

++++-+-	-----+---	-----	5804,5806 = 5806,5815
++++-----	--+-+---	-----	5804,5807 = 5807,5815
++++-----	--+-+---	-----	5802,5804 = 5802,5815
+++--+-	-----+---	-----	5801,5806 = 5806,5819
++-+-	-----+---	-----	5804,5805 = 5805,5815
++-+-	+----+---	-----	5804,5808 = 5808,5815
++-+-	-----+---	-----	5804,5819 = 5815,5819
++-+-	-+-+---	-----	5804,5809 = 5809,5815
++-+-	----+---	-----	5804,5810N = 5810N,5815
++-+-	-----+--	-----	5804,5811 = 5804,5820 = 5811,5815 = 5815,5820
++-+-	-----+--	-----	5804,5812 = 5812,5815
++-+-	-----+--	-----	5804,5813 = 5813,5815
++-+-	-----+--	+--+-	5804,5818 = 5815,5818
++-+-	-----+--	+----	5804,5816 = 5815,5816
++-+-	-----+--	-+---	5804,5817N = 5815,5817N
++-+-	-----+--	---+-	5804,5821 = 5815,5821
++-+-	-----+--	-----+	5804,5814 = 5814,5815
++-+-	-----+--	-----	5801,5804 = 5801,5815 = 5804,5815 = 5815,5815
++-+-	-----+--	-----	5805,5811 = 5805,5820
++-+-	-----+--	-----	5801,5805 = 5805,5805
++-+-	+----+---	-----	5801,5808 = 5808,5819
++-+-	-----+--	-----	5811,5819 = 5819,5820
++-+-	-----+--	-----	5801,5819 = 5819,5819
++-+-	-+-+---	-----	5809,5811 = 5809,5820
++-+-	-+-+---	-----	5801,5809 = 5809,5809
++-+-	----+---	-----	5810N,5811 = 5810N,5820
++-+-	----+---	-----	5801,5810N = 5810N,5810N
++-+-	-----+--	-----	5811,5812 = 5812,5820
++-+-	-----+--	-----	5811,5813 = 5813,5820
++-+-	-----+--	-+---	5811,5817N = 5817N,5820
++-+-	-----+--	---+-	5811,5821 = 5820,5821
++-+-	-----+--	-----+	5811,5814 = 5814,5820
++-+-	-----+--	-----	5801,5811 = 5801,5820
++-+-	-----+--	-----	5801,5812 = 5812,5812
++-+-	-----+--	-----	5801,5813 = 5813,5813
++-+-	-----+--	-+---	5801,5817N = 5817N,5817N
++-+-	-----+--	---+-	5801,5821 = 5821,5821
++-+-	-----+--	-----+	5801,5814 = 5814,5814
++-+-	-----+--	-----	5811,5811 = 5811,5820
++-+-	--+-+---	-----	5802,5807 = 5807,5807
++-+-	-----+--	+--+-	5816,5818 = 5818,5818

5801 = 580101-580102

## SPECIFICITY TABLE

### HLA-B\*58 SSP subtyping

Specificities and sizes of the PCR products of the 21 primer mixes used for HLA-B\*58 SSP subtyping

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-B*58 alleles	Other amplified HLA Class I alleles <sup>3</sup>
1 <sup>4</sup>	90 bp	800 bp	*580101-5802, 5805-5821	
2	150 bp	800 bp	*580101, 580102, 5804, 5805, 5809-5815, 5817N, 5819, 5821	*130101, 130102, 1306, 1307N, 1312, 1313, 1317, 1320, 132201-1323, 1325, 1326, 1410, 1502, 1513, 1520, 1521, 152501, 1536, 1544, 1562, 1577, 1580, 1585, 1588, 1589, 9506, 9512, 9521, 9539, 9544, 9554, 1822, 2719, 2730, 350101-350403, 3506-350803, 3510-3517, 3519-3521, 3523-3530, 3533-3536, 3538-3542, 3545-3550, 3552, 3554-3557, 3559, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3580-3585, 3590-3595, 370101-370105, 3703N-3706, 3708, 3710, 3711, 3713, 3714, 3942, 4028, 44020101-440205, 440208-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-446402, 4466, 4467, 4469, 4802, 5104, 5142, 5146, 5156, 530101-5306, 5308-5313, 5315-5318, 5514, 5609, 5611, 5612, 570101-5710, 5712, 5714-5719, 8301
3	240 bp	800 bp	*5802, 5806, 5807	*5711, <b>Cw*0317</b>
4 <sup>8</sup>	190 bp, 230 bp	1070 bp	*5804, 5815	
5 <sup>4</sup>	90 bp	1070 bp	*5805	*9524, 4077, 4087, 4814
6	170 bp	1070 bp	*5806, 5808, 5819	*070201-070207, 070209-0724, 0726-0747, 0749N, 0750, 0752-0772, 0775-0777, 0806, 0820, 1316, 1320, 1401-1410, 15010101-150104, 150106-1504, 1506-1519, 1521, 1523-1530, 1532-1540, 1542-1547, 1549, 1550, 1553, 1554, 1556-1558, 1560-1574,

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				1576-1582, 1585, 1587, 1589, 1590, 1592-1599, 9501-9504, 9506, 9508-9510, 9512, 9513, 9515-9522, 9525-9529, 9531-9535, 9537-9544, 9546-9550, 9552-9554, 9556-9561, 9563, 9564, 1815, 1819, 1821, 1830, 270401, 270402, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2725, 2740, 3511, 351401, 351402, 3521, 3543, 3544, 3558, 3567, 3579, 3586, 3599, 3707, 3713, 3810, 3918, 3935, 3936, 4005, 4015, 4016, 4023, 4026, 4028, 4032, 4051, 4095, 4098, 460101-4605, 4607N, 4608, 4610, 4612, 4614-4617, 4805, 4808, 4815, 490101-4905, 5001, 5002, 5004, 510101-5104, 5106, 5107, 5111N-5114, 5116-5118, 5121-512402, 5126-5130, 5132-5139, 5141N, 5143, 5145, 5146, 5148-5152, 5155-5165, 520101-5202, 5204-5209, 5211-5214, 5306, 5308, 5406, 550101-550104, 5503, 5505, 5509, 5511, 5514, 5515, 5517, 5521, 5524, 5525, 5528, 5529, 5531, 5533, 5603, 560501-5606, 5621, 5709, 5713, 7801-7803, 7805, 7806
<b>7<sup>4,5</sup></b>	80 bp	1070 bp	*5806, 5808, 5819	*1316, 1320, 1401-140202, 1403 <sup>w</sup> , 1404-1410, 1542, 1815, 3511, 3521, 3558, 3810, 3935, 4028, 4095, 490101, 4902-4905, 5001, 5002, 5004, 510101-5104, 5106, 5107, 5111N-5114, 5116-5118, 5121-512401, 5126-5128, 5129 <sup>w</sup> , 5130, 5132-5135, 5137-5139, 5141N, 5143, 5145, 5146, 5148-5153, 5155 <sup>w</sup> , 5156-5160, 5162-5165, 520101-5202, 5204-5209, 5211-5214, 5306, 5308, 550101-550104, 5503, 5505, 5509 <sup>w</sup> , 5511, 5514, 5515, 5517, 5524, 5525, 5528, 5529, 5531, 5533, 560501-5606, 5621, 7801-7803, 7805, 7806
<b>8</b>	250 bp	1070 bp	*5807	
<b>9</b>	155 bp	<b>800 bp</b>	*5808	
<b>10</b>	270 bp	<b>800 bp</b>	*5809	*1410, 1822, 3521, 3524, 3581, 3704, 4028, 5104, 5146, 5156, 5302, 5306, 5714

<b>11</b>	205 bp	1070 bp	*5802, 5807	5711, <b>A*2464<sup>w</sup>, Cw*0106<sup>w</sup></b>
<b>12</b>	235 bp	1070 bp	*5810N	
<b>13<sup>9</sup></b>	165 bp	1070 bp	*580101- 5802, 5804- 5810N, 5812- 5819, 5821	*1520, 350101-3530, 3532-3542, 3544-3556, 3558, 3559, 3561-3572, 3574-3578, 3580-3586, 3588-3595, 3597, 3599, 4802, 510101-512402, 5126-5141N, 5143-5146, 5148-5165, 520101-520602, 5208-5214, 530101- 5317, 560501, 560502, 5621, 7801- 7806, 8103, 8301 <sup>?</sup>
<b>14<sup>6,9</sup></b>	165 bp	1070 bp	*5811, 5820	*070201-070207, 070209-0757, 0759- 0772, 0775-0777, 080101-0838, 0840, 130101-1304, 1306-1323, 1325, 1326, 1401-1410, 15010101-150104, 150106-1519, 1521, 1523-1540, 1542- 1558, 1560-1599, 9501, 9503-9529, 9531-9561, 9563, 9564, 180101-1815, 1817N-1833, 1835, 2701-2721, 2723- 2746, 2750-2752, 3531, 3535, 3543, 3546, 3557, 3560, 3579, 3587, 370101-3714, 380101-3819, 39010101, 39010102L, 390103-3920, 3922-3945, 3947-3950, 400101-4016, 4018-4040, 4042-4098, 4101-4108, 4201, 4202, 4204-4212, 44020101- 440205, 440208-4465, 4467, 4469, 4501-4509, 460101-4619, 4621, 47010101-4705, 480101, 480102, 480301-4819, 490101-4905, 5001, 5002, 5004, 5142, 5207, 5401-5419, 550101-5505, 5507-5535, 5601-5604, 5606-5620, 5622-5624, 570101-5719, 5901-5905, 670101-6702, 8101, 8102, 8104N, 8201, 8202, 8301 <sup>?</sup>
<b>15</b>	155 bp	1070 bp	*5812	
<b>16</b>	145 bp	1070 bp	*5813	
<b>17<sup>7</sup></b>	375 bp	1070 bp	*5816, 5818	
<b>18</b>	140 bp	1070 bp	*5817N	
<b>19<sup>4</sup></b>	95 bp	<b>800 bp</b>	*5818	*0709, 0711, 0717, 0828, 0835, 0837, 1507, 1555, 1568, 9526, 1814, 3505, 3551, 3558, 3566, 3572, 3589, 3597, 4003, 4209, 4612, 4814, 5314
<b>20</b>	170 bp	1070 bp	*5821	
<b>21</b>	440 bp	1070 bp	*5814	

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<sup>1</sup>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-B\*58 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.

<sup>2</sup>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\*58 subtyping.

In addition, wells number 2, 3, 9, 10 and 19 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.

<sup>3</sup>Due to the sharing of sequence motifs between HLA-B alleles some non-HLA-B\*58 alleles will be amplified by primer mixes 2, 3, 5, 6, 7, 10, 11, 13, 14 and 19. In addition, the Cw\*0317 allele will be amplified by primer mix 3, and the A\*2464 and Cw\*0106 alleles will be weakly amplified by primer mix 11.

<sup>4</sup>Short specific PCR fragments are less intense and not as sharp as longer specific bands.

<sup>5</sup>Primer mix 7 may give rise to unspecific amplifications.

<sup>6</sup>Primer mix 14 has a tendency of primer dimer formation.

<sup>7</sup>Primer mixes 17 has a tendency of giving rise to nonspecific amplifications.

<sup>8</sup>Primer mix 4: Specific PCR fragment of 190 bp in the B\*5804 allele. Specific PCR fragment of 230 bp in the B\*5815 allele.

<sup>9</sup>Primer mixes 13 and 14: For many HLA-B alleles fourth exon nucleotide sequences are not available. We assume that unknown sequences in the fourth exon are conserved within allelic groups.

‘?’ , nucleotide sequence information not available for the primer matching sequence.

‘w’, might be weakly amplified.

## INTERPRETATION TABLE

### HLA-B\*58 SSP subtyping

#### Amplification patterns of the B\*5801 to B\*5821 alleles

	Well <sup>5</sup>											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	90	150	240	190	90	170	80	250	155	270	205	235
PCR product(s)				230								
Length of int.	800	800	800	1070	1070	1070	1070	1070	800	800	1070	1070
pos. control <sup>1</sup>												
5'-primer(s) <sup>2</sup>	209 5'-ggA <sup>3</sup>	355 5'-TCA <sup>3</sup>	361 5'-AgT <sup>3</sup>	106 5'-CCA <sup>3</sup>	463 5'-TgA <sup>3</sup>	527 5'-TgA <sup>3</sup>	486 5'-ACC <sup>3</sup>	361 5'-AgT <sup>3</sup>	141 5'-ATT <sup>3</sup>	355 5'-TCA <sup>3</sup>	361 5'-AgT <sup>3</sup>	367 5'-T.T <sup>3</sup>
3'-primer(s) <sup>3</sup>	256 5'-CCC <sup>3</sup>	463 5'-gCT <sup>3</sup>	559 5'-CAg <sup>3</sup>	256 5'-CTC <sup>3</sup>	512 5'-CCA <sup>3</sup>	3 <sup>rd</sup>   5'-TAT <sup>3</sup>	527 5'-CCT <sup>3</sup>	572 5'-gCg <sup>3</sup>	256 5'-CCC <sup>3</sup>	583 5'-gTg <sup>3</sup>	527 5'-CCA <sup>3</sup>	559 5'-CAg <sup>3</sup>
				296 5'-CTg <sup>3</sup>								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-B allele <sup>4</sup>												
*580101, 580102	1	2										
*5802	1		3								11	
*5804		2		4								
*5805	1	2			5							
*5806	1		3			6	7					
*5807	1		3					8			11	
*5808	1					6	7		9			
*5809	1	2								10		
*5810N	1	2										12
*5811	1	2										
*5812	1	2										
*5813	1	2										
*5814	1	2										
*5815	1	2		4								
*5816	1											
*5817N	1	2										
*5818	1											
*5819	1	2				6	7					
*5820	1											
*5821	1	2										
Well No.	1	2	3	4	5	6	7	8	9	10	11	12



<b>INTERPRETATION TABLE</b>									
<b>HLA-B*58 SSP subtyping</b>									
<b>Amplification patterns of the B*5801 to B*5821 alleles</b>									
<b>Well<sup>5</sup></b>									
13	14	15	16	17	18	19	20	21	
165	165	155	145	375	140	95	170	440	<b>Length of spec. PCR product(s)</b>
1070	1070	1070	1070	1070	1070	800	1070	1070	<b>Length of int. pos. control<sup>1</sup></b>
652	652	142	454	281	209	363	209	206	<b>5'-primer(s)<sup>2</sup></b>
5'-CCg <sup>3</sup>	5'-CCA <sup>3</sup>	5'-TCT <sup>3</sup>	5'-ACA <sup>3</sup>	5'-CTC <sup>3</sup>	5'-ggA <sup>3</sup>	5'-AgC <sup>3</sup>	5'-ggA <sup>3</sup>	5'-gAC <sup>3</sup>	
774	774	256	559	369	309	419	340	362	<b>3'-primer(s)<sup>3</sup></b>
5'-ggT <sup>3</sup>	5'-ggT <sup>3</sup>	5'-CCC <sup>3</sup>	5'-CAg <sup>3</sup>	5'-CCg <sup>3</sup>	5'-A.C <sup>3</sup>	5'-Cgg <sup>3</sup>	5'-ggT <sup>3</sup>	5'-TCA <sup>3</sup>	
13	14	15	16	17	18	19	20	21	<b>Well No.</b>
									<b>HLA-B allele<sup>4</sup></b>
13									<b>*580101, 580102</b>
13									<b>*5802</b>
13									<b>*5804</b>
13									<b>*5805</b>
13									<b>*5806</b>
13									<b>*5807</b>
13									<b>*5808</b>
13									<b>*5809</b>
13									<b>*5810N</b>
	14								<b>*5811</b>
13		15							<b>*5812</b>
13			16						<b>*5813</b>
13								21	<b>*5814</b>
13									<b>*5815</b>
13				17					<b>*5816</b>
13					18				<b>*5817N</b>
13				17		19			<b>*5818</b>
13									<b>*5819</b>
	14								<b>*5820</b>
13							20		<b>*5821</b>
13	14	15	16	17	18	19	20	21	<b>Well No.</b>

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Length of spec.	90	150	240	190	90	170	80	250	155	270	205	235
PCR product(s)				230								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*070201-070207, 070209-0708, 0710, 0712-0716, 071801-0724, 0726-0747, 0749N, 0750, 0752-0757, 0759-0772, 0775-0777, 0806, 0820, 15010101-150104, 150106-150108, 150301, 1504, 1506, 1508-1512, 1514-1519, 1523, 1524, 1526N-1530, 1532-1535, 1537-1540, 1543, 1545-1547, 1549, 1550, 1553, 1554, 1556-1558, 1560, 1561, 1563-1567, 1569-1574, 1576, 157801, 1579N, 1581, 1582, 1587, 1590, 1592-1599, 9501, 9503, 9504, 9508-9510, 9513, 9515-9520, 9522, 9525, 9527-9529, 9531-9535, 9537, 9538, 9540-9543, 9546-9550, 9552, 9553, 9556-9561, 9563, 9564, 1819, 1821, 1830, 270401, 270402, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2725, 2740, 3543, 3579, 3707, 3918, 3936, 4005, 4015, 4016, 4023, 4026, 4032, 4051, 4098, 460101-4605, 4607N, 4608, 4610, 4614-4617, 4805, 4808, 4815, 490102, 5406, 5521, 5603, 5713						6						
*0709, 0711, 0717, 1507, 1568, 9526, 4612						6						
*0725, 0748, 0751, 080101-0805, 0807-0819N, 0821-0827, 0829-0834, 0836, 0838, 0840, 130201-1304, 1308Q-1311, 1314, 1315, 1318, 1319, 1321, 1505, 1531, 1548, 1551, 1552, 1575, 1583, 1584, 1586, 1591, 9505, 9507, 9511N, 9514, 9523, 9536, 9545, 9551, 9555, 180101-1813, 1817N, 1818, 1820, 1823N-1829, 1831-1833, 1835, 2701-2703, 270502-270510, 2707-2709, 2711-2714, 2716, 2717, 2723, 2726-2729, 2731-2739, 2741-2746, 2750-2752, 3531, 3560, 3587, 3702, 3709, 3712, 380101-3809, 3811-3819, 39010101, 39010102L, 390103-3917, 391901-3920, 3922-3934, 3937-3941, 3943-3945, 3947-3950,												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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165	165	155	145	375	140	95	170	440	Length of spec. PCR product(s)
13	14	15	16	17	18	19	20	21	Well No.
	14								*070201-070207, 070209-0708, 0710, 0712-0716, 071801-0724, 0726-0747, 0749N, 0750, 0752-0757, 0759-0772, 0775-0777, 0806, 0820, 15010101-150104, 150106-150108, 150301, 1504, 1506, 1508-1512, 1514-1519, 1523, 1524, 1526N-1530, 1532-1535, 1537-1540, 1543, 1545-1547, 1549, 1550, 1553, 1554, 1556-1558, 1560, 1561, 1563-1567, 1569-1574, 1576, 157801, 1579N, 1581, 1582, 1587, 1590, 1592-1599, 9501, 9503, 9504, 9508-9510, 9513, 9515-9520, 9522, 9525, 9527-9529, 9531-9535, 9537, 9538, 9540-9543, 9546-9550, 9552, 9553, 9556-9561, 9563, 9564, 1819, 1821, 1830, 270401, 270402, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2725, 2740, 3543, 3579, 3707, 3918, 3936, 4005, 4015, 4016, 4023, 4026, 4032, 4051, 4098, 460101-4605, 4607N, 4608, 4610, 4614-4617, 4805, 4808, 4815, 490102, 5406, 5521, 5603, 5713
	14					19			*0709, 0711, 0717, 1507, 1568, 9526, 4612
	14								*0725, 0748, 0751, 080101-0805, 0807-0819N, 0821-0827, 0829-0834, 0836, 0838, 0840, 130201-1304, 1308Q-1311, 1314, 1315, 1318, 1319, 1321, 1505, 1531, 1548, 1551, 1552, 1575, 1583, 1584, 1586, 1591, 9505, 9507, 9511N, 9514, 9523, 9536, 9545, 9551, 9555, 180101-1813, 1817N, 1818, 1820, 1823N-1829, 1831-1833, 1835, 2701-2703, 270502-270510, 2707-2709, 2711-2714, 2716, 2717, 2723, 2726-2729, 2731-2739, 2741-2746, 2750-2752, 3531, 3560, 3587, 3702, 3709, 3712, 380101-3809, 3811-3819, 39010101, 39010102L, 390103-3917, 391901-3920, 3922-3934, 3937-3941, 3943-3945, 3947-3950,
13	14	15	16	17	18	19	20	21	Well No.

Lot No.: **35G**

Lot-specific Information

www.olerup.com

Length of spec.	90	150	240	190	90	170	80	250	155	270	205	235
PCR product(s)				230								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
400101-400205, 4004, 40060101-401403, 4018-4022N, 4024, 4025, 4027, 4029-4031, 4033-4040, 4042-4050, 4052-4076, 4078-4086, 4088-4094, 4096, 4097, 4101-4108, 4201, 4202, 4204-4208, 4210-4212, 4415, 4418, 4420, 4431, 4441, 4447, 4453, 4454, 4465, 4501-4509, 4606, 4609, 4611, 461301, 461302, 4618, 4619, 4621, 47010101-4705, 480101, 480102, 480301-4804, 4806, 4807, 4809-4813, 4816-4819, 5401-5405N, 5407-5419, 550201-550203, 5504, 5507, 5508, 5510, 5512, 5513, 5516, 5518-5520, 5522, 5523, 5526, 5527, 5530, 5532, 5534, 5535, 5601, 5602, 5604, 5607, 5608, 5610, 5613-5620, 5622-5624, 5901-5905, 670101-6702, 8101, 8102, 8104N, 8201, 8202												
*0758, 9502						6						
*0828, 0835, 0837, 1555, 1814, 4003, 4209												
*130101, 130102, 1306, 1307N, 1312, 1313, 1317, 132201-1323, 1325, 1326, 1588, 2719, 2730, 3557, 370101-370105, 3703N, 3705, 3706, 3708, 3710, 3711, 3714, 3942, 44020101-440205, 440208-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-446402, 4467, 4469, 5142, 5609, 5611, 5612, 570101-5708, 5710, 5712, 5715-5719		2										
*1316, 1401-140202, 1404-1409, 1542, 1815, 3810, 3935, 4095, 490101, 4902-4905, 5001, 5002, 5004, 5207, 550101-550104, 5503, 5505, 5511, 5515, 5517, 5524, 5525, 5528, 5529, 5531, 5533, 5606						6	7					
*1320, 5514		2				6	7					
*1403, 5509						6	w					
*1410, 4028		2				6	7		10			
*1502, 1513, 1521, 152501, 1536, 1544, 1562, 1577, 1580, 1585, 1589, 9506, 9512, 9521, 9539, 9544, 9554, 3713, 5709		2				6						
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **35G**

Lot-specific Information

www.olerup.com

165	165	155	145	375	140	95	170	440	Length of spec. PCR product(s) Well No.
13	14	15	16	17	18	19	20	21	
	14								400101-400205, 4004, 40060101-401403, 4018-4022N, 4024, 4025, 4027, 4029-4031, 4033-4040, 4042-4050, 4052-4076, 4078-4086, 4088-4094, 4096, 4097, 4101-4108, 4201, 4202, 4204-4208, 4210-4212, 4415, 4418, 4420, 4431, 4441, 4447, 4453, 4454, 4465, 4501-4509, 4606, 4609, 4611, 461301, 461302, 4618, 4619, 4621, 47010101-4705, 480101, 480102, 480301-4804, 4806, 4807, 4809-4813, 4816-4819, 5401-5405N, 5407-5419, 550201-550203, 5504, 5507, 5508, 5510, 5512, 5513, 5516, 5518-5520, 5522, 5523, 5526, 5527, 5530, 5532, 5534, 5535, 5601, 5602, 5604, 5607, 5608, 5610, 5613-5620, 5622-5624, 5901-5905, 670101-6702, 8101, 8102, 8104N, 8201, 8202
									*0758, 9502
	14					19			*0828, 0835, 0837, 1555, 1814, 4003, 4209
									*130101, 130102, 1306, 1307N, 1312, 1313, 1317, 132201-1323, 1325, 1326, 1588, 2719, 2730, 3557, 370101-370105, 3703N, 3705, 3706, 3708, 3710, 3711, 3714, 3942, 44020101-440205, 440208-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-446402, 4467, 4469, 5142, 5609, 5611, 5612, 570101-5708, 5710, 5712, 5715-5719
	14								*1316, 1401-140202, 1404-1409, 1542, 1815, 3810, 3935, 4095, 490101, 4902-4905, 5001, 5002, 5004, 5207, 550101-550104, 5503, 5505, 5511, 5515, 5517, 5524, 5525, 5528, 5529, 5531, 5533, 5606
	14								*1320, 5514
	14								*1403, 5509
	14								*1410, 4028
	14								*1502, 1513, 1521, 152501, 1536, 1544, 1562, 1577, 1580, 1585, 1589, 9506, 9512, 9521, 9539, 9544, 9554, 3713, 5709
13	14	15	16	17	18	19	20	21	Well No.

Lot No.: **35G**

Lot-specific Information

www.olerup.com

Length of spec.	90	150	240	190	90	170	80	250	155	270	205	235
PCR product(s)				230								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*1520, 350101-350403, 3506-350803, 3510, 3512, 3513, 3515-3517, 3519-352002, 3523, 3525-3530, 3533, 3534, 3536, 3538-3542, 3545, 3547-3550, 3552, 3554-3556, 3559, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3580, 3582-3585, 3590-3595, 4802, 530101-530104, 5303-5305, 5309-5313, 5315-5317		2										
*1822, 3704, 5714		2								10		
*3505, 3551, 3566, 3572, 3589, 3597, 5314												
*350901, 350902, 3518, 3522, 3532, 3537, 3553N, 3564, 356801, 356802, 3575, 3588, 5105, 5108-5110, 5115, 5119, 5120, 5131, 5140, 5144N, 5154, 5203, 5210, 5307, 7804, 8103												
*3511, 5308		2				6	7					
*351401, 351402		2				6						
*3521, 5104, 5146, 5156, 5306		2				6	7			10		
*3524, 3581, 5302		2								10		
*3535, 3546		2										
*3544, 3567, 3586, 3599, 512402, 5136, 5161						6						
*3558						6	7					
* 9524, 4077, 4087					5							
*4466, 5318		2										
*4814					5							
*510101-5103, 5106, 5107, 5111N-5114, 5116-5118, 5121-512401, 5126-5128, 5130, 5132-5135, 5137-5139, 5141N, 5143, 5145, 5148-5152, 5157-5160, 5162-5165, 520101-5202, 5204-520602, 5208, 5209, 5211-5214, 560501, 560502, 5621, 7801-7803, 7805, 7806						6	7					
*5129, 5155						6	w					
*5153							7					
*5711			3								11	
*8301		2										
A*2464, Cw*0106											w	
Cw*0317			3									
HLA-B allele <sup>4</sup>												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **35G**

Lot-specific Information

www.olerup.com

165	165	155	145	375	140	95	170	440	Length of spec. PCR product(s) Well No.
13	14	15	16	17	18	19	20	21	
13									*1520, 350101-350403, 3506-350803, 3510, 3512, 3513, 3515-3517, 3519-352002, 3523, 3525-3530, 3533, 3534, 3536, 3538-3542, 3545, 3547-3550, 3552, 3554-3556, 3559, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3580, 3582-3585, 3590-3595, 4802, 530101-530104, 5303-5305, 5309-5313, 5315-5317
	14								*1822, 3704, 5714
13						19			*3505, 3551, 3566, 3572, 3589, 3597, 5314
13									*350901, 350902, 3518, 3522, 3532, 3537, 3553N, 3564, 356801, 356802, 3575, 3588, 5105, 5108-5110, 5115, 5119, 5120, 5131, 5140, 5144N, 5154, 5203, 5210, 5307, 7804, 8103
13									*3511, 5308
13									*351401, 351402
13									*3521, 5104, 5146, 5156, 5306
13									*3524, 3581, 5302
13	14								*3535, 3546
13									*3544, 3567, 3586, 3599, 512402, 5136, 5161
13						19			*3558
	14								* 9524, 4077, 4087
									*4466, 5318
	14					19			*4814
13									*510101-5103, 5106, 5107, 5111N-5114, 5116-5118, 5121-512401, 5126-5128, 5130, 5132-5135, 5137-5139, 5141N, 5143, 5145, 5148-5152, 5157-5160, 5162-5165, 520101-5202, 5204-520602, 5208, 5209, 5211-5214, 560501, 560502, 5621, 7801-7803, 7805, 7806
13									*5129, 5155
13									*5153
	14								*5711
?	?								*8301
									A*2464, Cw*0106
									Cw*0317
									HLA-B allele <sup>4</sup>
13	14	15	16	17	18	19	20	21	Well No.

Lot No.: **35G**

Lot-specific Information

[www.olerup.com](http://www.olerup.com)

<sup>1</sup>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\*58 subtyping.

In addition, wells number 2, 3, 9, 10 and 19 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.

<sup>2</sup>The nucleotide position, in the 2<sup>nd</sup>, 3<sup>rd</sup> or 4<sup>th</sup> exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>3</sup>The nucleotide position, in the 2<sup>nd</sup>, 3<sup>rd</sup> or 4<sup>th</sup> exon or the 3<sup>rd</sup> 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](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>4</sup>The B\*5803 allele was never assigned.

<sup>5</sup>Primer mix 4: Specific PCR fragment of 190 bp in the B\*5804 allele. Specific PCR fragment of 230 bp in the B\*5815 allele.

'w', might be weakly amplified.

'?', nucleotide sequence information not available for the primer matching sequence.



CELL LINE VALIDATION SHEET																			
HLA-B*58 SSP subtyping kit																			
			Prod. No.:	Well															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	IHWC cell line	B*		200851501	200618102	200618103	200738004	200618105	200618106	200964307	200618108	200964309	200618110	200618111	200618112	200618113	200964314	200624685	200624716
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	*440301		-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-
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	*440301		-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-
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	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-
49	9366 DAUDI	*5801	*5802	+	+	+	-	-	-	-	-	-	-	+	-	+	-	-	-

<b>CELL LINE VALIDATION SHEET</b>										
<b>HLA-B*58 SSP subtyping kit</b>										
					<b>Well</b>					
					17	18	19	20	21	
					Prod. No.:	200851517	200851518	200851519	200964322	200851521
	<b>IHWC cell line</b>		<b>B*</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	*440301		-	-	-	-	-	
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	*440301		-	-	-	-	-	
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	-	-	-	-	-	
49	9366	DAUDI	*5801	*5802	-	-	-	-	-	

## CERTIFICATE OF ANALYSIS

### Olerup SSP<sup>®</sup> HLA-B\*58 SSP

Product number: 101.568-06 – including *Taq* polymerase  
Lot number: 35G  
Expiry date: 2011-October-01  
Number of tests: 6  
Number of wells per test: 21

#### Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2008-515-01	9	2009-643-09	17	2008-515-17
2	2006-181-02	10	2006-181-10	18	2008-515-18
3	2006-181-03	11	2006-181-11	19	2008-515-19
4	2007-380-04	12	2006-181-12	20	2009-643-22
5	2006-181-05	13	2006-181-13	21	2008-515-21
6	2006-181-06	14	2009-643-14		
7	2009-643-07	15	2006-247-15		
8	2006-181-08	16	2006-247-16		

The specificity of each primer solution of the kit has been tested against 49 well characterized cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 4, 5, 8 to 10, 12, 15 to 18, 20 and 21 were available. The specificities of the primers in primer solutions 5, 8, 9, 10, 15 and 17 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 4, 18, 20 and 21 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 12 and 16 it was only possible to test the 3'-primers, the 5'-primers were 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

## Declaration of Conformity

**Product name:** *Olerup* SSP<sup>®</sup> HLA-B\*58  
**Product number:** 101.568-06  
**Lot number:** 35G

**Intended use:** HLA-B\*58 high resolution histocompatibility testing

**Manufacturer:** *Olerup* SSP AB  
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**Phone:** +46-8-717 88 27  
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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  
2009-October-23

Olle Olerup  
Managing Director







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