

Lot No.: **13F**

Lot-specific Information

***Olerup* SSP[®] HLA-B*58**

Product number:	101.568-06u – without <u><i>Taq</i></u> polymerase
Lot number:	13F
Expiry date:	2010-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. 13F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP[®] 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[®] HLA-B*58 lot was made (Lot No. Y35).

Five wells have been added to the HLA-B*58 kit,
wells **17 to 21**.

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

Well	5'-primer	3'-primer	rationale
1	-	-	Change of control primer pair to avoid primer dimer formation.
7	-	-	Change of control primer pair to avoid primer dimer formation.
9	Removed	Removed	The primer pair for the B*5814 allele has been removed.
17	New	New	New primer pair for the B*5816 and 5818 alleles.
18	New	New	New primer pair for the B*5817N and allele.
19	New	New	New primer pair for the B*5818 and allele.
20	New	New	New primer pair for the B*5819 allele.
21	New	New	New primer pair for the B*5814 allele.

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PRODUCT DESCRIPTION

HLA-B*58 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*5801 to B*5819 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. '13F'.

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, 19 and 20 amplify many non-HLA-B*58 alleles. In addition, the Cw*0317 allele will be amplified by primer mix 3.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*58 alleles, i.e. **B*5801 to B*5819**, recognized by the HLA Nomenclature Committee in July 2008¹ 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.

¹HLA-A alleles listed on the IMGT/HLA web page 2008-July-11, release 2.22.0, www.ebi.ac.uk/imgt/hla.

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RESOLUTION IN HOMO- AND HETEROZYGOTES

The eighteen HLA-B*58 alleles can be combined in 153 homozygous and heterozygous combinations. Fifty-eight 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 = 5811, 5815
++-+---	-----+-+	-----	5804, 5812 = 5812, 5815
++-+---	-----++-	-----	5804, 5813 = 5813, 5815
++-+---	-----+--	+--+-	5804, 5818 = 5815, 5818
++-+---	-----+--	+-----	5804, 5816 = 5815, 5816
++-+---	-----+---	-+---	5804, 5817N = 5815, 5817N
++-+---	-----+---	-----+	5804, 5814 = 5814, 5815
++-+---	-----+---	-----	5801, 5804 = 5801, 5815 = 5804, 5815 = 5815, 5815
++-+---	-----+---	-----	5801, 5805 = 5805, 5805
++-++-	+---+---	---+-	5801, 5808 = 5808, 5819
++-++-	-----+---	---+-	5801, 5819 = 5819, 5819
++-----	-+-+---	-----	5801, 5809 = 5809, 5809
++-----	---++---	-----	5801, 5810N = 5810N, 5810N
++-----	-----+-+	-----	5801, 5812 = 5812, 5812
++-----	-----++-	-----	5801, 5813 = 5813, 5813
++-----	-----+--	-+---	5801, 5817N = 5817N, 5817N
++-----	-----+---	-----+	5801, 5814 = 5814, 5814
+--+-----+	--+-+---	-----	5802, 5807 = 5807, 5807
+-----	-----+---	+--+-	5816, 5818 = 5818, 5818

5801 = 580101-580102

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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 ¹	Size of control band ²	Amplified HLA-B*58 alleles	Other amplified HLA Class I alleles ³
1⁴	90 bp	800 bp	580101-5802, 5805-5819	
2	150 bp	800 bp	580101-580102, 5804, 5805, 5809-5815, 5817, 5819	1301, 1306, 1307N, 1312, 1313, 1317, 1320, 1322, 1502, 1513, 1520, 1521, 1525, 1536, 1544, 1562, 1577, 1580, 1585, 1588, 1589, 9506, 9512, 9521, 9539, 9544, 1822, 2719, 2730, 350101-350403, 3506-350803, 3510-3517, 3519-3521, 3523-3530, 3533-3536, 3538-3542, 3545-3550, 3552-3557, 3559, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3580-3585, 3590-3594, 370101-370104, 3703N-3706, 3708, 3710, 3711, 3713, 3942, 4028, 44020101-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-446402, 4802, 5104, 5142, 5146, 5156, 530101-5306, 5308-5313, 5315, 5316, 5514, 5609, 5611, 5612, 570101-5710, 5712, 5714-5717, 8301
3	240 bp	800 bp	5802, 5806, 5807	5711, Cw*0317
4⁸	190, 230 bp	1070 bp	5804, 5815	
5⁴	90 bp	1070 bp	5805	9524, 4077, 4087, 4814
6	170 bp	1070 bp	5806, 5808, 5819	070201-0724, 0726-0747, 0749N, 0750, 0752-0765, 0806, 0820, 1316, 1320, 1401-1409, 15010101-150104, 150106-1504, 1506-1519, 1521, 1523-1530, 1532-1540, 1542-1547, 1549, 1550, 1553, 1554, 1556-1558,

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				1560-1574, 1576-1582, 1585, 1587, 1589, 1590, 1592-1599, 9501-9504, 9506, 9508-9510, 9512, 9513, 9515-9522, 9525-9529, 9531-9535, 9537-9544, 9546-9549N, 1815, 1819, 1821, 270401-270402, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2725, 2740, 3511, 351401-351402, 3521, 3543, 3544, 3558, 3567, 3579, 3586, 3707, 3713, 3810, 3918, 3935, 3936, 4005, 4015, 4016, 4023, 4026, 4028, 4032, 4051, 460101-4605, 4607N, 4608, 4610, 4612, 4614-4616, 4805, 4808, 4815, 4901-4905, 5001, 5002, 5004, 510101-5104, 5106, 5107, 5111N-5114, 5116-5118, 5121-512402, 5126-5130, 5132-5139, 5141N, 5143, 5145, 5146, 5148-5152, 5155-5157, 520101-5202, 5204-5209, 5211-5214, 5306, 5308, 5406, 550101-550104, 5503, 5505, 5509, 5511, 5514, 5515, 5517, 5521, 5524, 5525, 5528, 5529, 5531, 5603, 560501-5606, 5621, 5709, 5713, 7801-7803, 7805, 7806
7^{4,5}	80 bp	1070 bp	5806, 5808, 5819	1316, 1320, 1401-140202, 1403 ^{weakly} , 1404-1409, 1542, 1815, 3511, 3521, 3558, 3810, 3935, 4028, 4901-4905, 5001, 5002, 5004, 510101-5104, 5106, 5107, 5111N-5114, 5116-5118, 5121-512401, 5126-5128, 5129 ^{weakly} , 5130, 5132-5135, 5137-5139, 5141N, 5143, 5145, 5146, 5148-5153, 5155 ^{weakly} , 5156, 5157, 520101-5202, 5204-5209, 5211-5214, 5306, 5308, 550101-550104, 5503, 5505, 5509 ^{weakly} , 5511, 5514, 5515, 5517, 5524, 5525, 5528, 5529, 5531, 560501-5606, 5621, 7801-7803, 7805, 7806
8	250 bp	1070 bp	5807	
9	155 bp	800 bp	5808	
10	270 bp	800 bp	5809	1822, 3521, 3524, 3581, 3704,

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				4028, 5104, 5146, 5156, 5302, 5306, 5714
11	205 bp	1070 bp	5802, 5807	5711
12	235 bp	1070 bp	5810N	
13^{8,9}	165 bp	1070 bp	580101-5802, 5804-5810N, 5812-5819	1520, 350101-3530, 3532-3542, 3544, 3545, 3547-3556, 3558, 3559, 3561-3572, 3574-3578, 3580-3586, 3588, 3590-3594, 4802, 510101-512402, 5126-5146, 5148-5157, 520101-520602, 5208-5214, 530101-5316, 5621, 7801-7806, 8103
14^{6,7,9}	165 bp	1070 bp	5811	070201-0757, 0759-0766, 080101-0836, 1301-1304, 1306-1322, 1401-1409, 15010101-150104, 150106-1519, 1521, 1523-1540, 1542-1558, 1560-1599, 9501, 9503-9529, 9531-9549N, 180101-1815, 1817N-1829, 2701-270402, 270502-2721, 2723-2743, 3531, 3543, 3546, 3557, 3560, 3579, 3587, 370101-3714, 380101-3817, 39010101-39010102L, 390103-3920, 3922-3945, 400101-4016, 4018-4040, 4042-4092, 4101-4108, 4201, 4202, 4204-4209, 44020101-446402, 4501-4509, 460101-4616, 47010101-4705, 480101-480102, 480301-4819, 4901-4905, 5001, 5002, 5004, 5207, 5401-5415, 550101-5505, 5507-5532, 5601-5620, 5622, 5623, 570101-5717, 5901-5903, 670101-6702, 8101, 8102, 8104, 8201, 8202, 8301
15	155 bp	1070 bp	5812	
16	145 bp	1070 bp	5813	
17⁶	370 bp	1070 bp	5816, 5818	
18	140 bp	1070 bp	5817N	
19⁴	95 bp	800 bp	5818	0709, 0711, 0717, 0828, 0835, 1507, 1555, 1568, 9526, 1814, 3505, 3551, 3558, 3566, 3572, 4003, 4209, 4612, 4814, 5314
20	530 bp	1070 bp	5806, 5808, 5819	1516-151702, 1567, 1595
21	440 bp	1070 bp	5814	

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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*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.

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*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.

³Due 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, 19 and 20. In addition, the Cw*0317 allele will be amplified by primer mix 3.

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

⁵Primer mix 7 has a tendency of giving rise to weak amplifications.

⁶Primer mixes 14 and 17 have a tendency of giving rise to nonspecific amplifications.

⁷Primer mix 14 has a tendency of primer dimer formation.

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

⁹Primer 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.

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INTERPRETATION TABLE												
HLA-B*58 SSP subtyping												
Amplification patterns of the B*5801 to B*5819 alleles												
	Well ⁵											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product(s)	90	150	240	190	90	170	80	250	155	270	205	235
Length of int.	800	800	800	1070	1070	1070	1070	1070	800	800	1070	1070
pos. control ¹												
5'-primer(s) ²	209	355	361	106	463	527	486	361	141	355	361	366
	5'-ggA ³	5'-TCA ³	5'-AgT ³	5'-CCA ³	5'-TgA ³	5'-TgA ³	5'-ACC ³	5'-AgT ³	5'-ATT ³	5'-TCA ³	5'-AgT ³	5'-ATT ³
3'-primer(s) ³	256	463	559	256	512	3 rd	527	572	256	585	527	559
	5'-CCC ³	5'-gCT ³	5'-CAg ³	5'-CTC ³	5'-CCA ³	5'-TAT ³	5'-CCT ³	5'-gCg ³	5'-CCC ³	5'-gTg ³	5'-CCA ³	5'-CAg ³
				296								
				5'-CTg ³								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-B allele ⁴												
*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					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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INTERPRETATION TABLE									
HLA-B*58 SSP subtyping									
Amplification patterns of the B*5801 to B*5819 alleles									
Well ⁵									
13	14	15	16	17	18	19	20	21	
165	165	155	145	370	140	95	530	440	Length of spec. PCR product(s)
1070	1070	1070	1070	1070	1070	800	1070	1070	Length of int.
									pos. control ¹
652	652	142	454	281	209	363	281	206	5'-primer(s) ²
5'-CCg ^{3'}	5'-CCA ^{3'}	5'-TCT ^{3'}	5'-ACA ^{3'}	5'-CTC ^{3'}	5'-ggA ^{3'}	5'-AgC ^{3'}	5'-CTC ^{3'}	5'-gAC ^{3'}	
774	774	256	559	369	309	419	527	362	3'-primer(s) ³
5'-ggT ^{3'}	5'-ggT ^{3'}	5'-CCC ^{3'}	5'-CAG ^{3'}	5'-CCg ^{3'}	5'-A.C ^{3'}	5'-Cgg ^{3'}	5'-CCT ^{3'}	5'-TCA ^{3'}	
13	14	15	16	17	18	19	20	21	Well No.
									HLA-B allele ⁴
13									*580101-580102
13									*5802
13									*5804
13									*5805
13							20		*5806
13									*5807
13							20		*5808
13									*5809
13									*5810N
	14								*5811
13		15							*5812
13			16						*5813
13								21	*5814
13									*5815
13				17					*5816
13					18				*5817N
13				17		19			*5818
13							20		*5819
13	14	15	16	17	18	19	20	21	Well No.

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Length of spec.	90	150	240	190	90	170	80	250	155	270	205	235
PCR product				230								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*070201-0708, 0710, 0712-0716, 071801-0724, 0726-0747, 0749N, 0750, 0752-0757, 0759-0766, 0806, 0820, 15010101-150104, 150106-150108, 1503, 1504, 1506, 1508-1512, 1514, 1515, 1518, 1519, 1523, 1524, 1526N-1530, 1532-1535, 1537-1540, 1543, 1545-1547, 1549, 1550, 1553, 1554, 1556-1558, 1560, 1561, 1563-1566, 1569-1574, 1576, 1578, 1579N, 1581, 1582, 1587, 1590, 1592-1594N, 1596-1599, 9501, 9503, 9504, 9508-9510, 9513, 9515-9520, 9522, 9525, 9527-9529, 9531-9535, 9537, 9538, 9540-9543, 9546-9549N, 1819, 1821, 270401-270402, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2725, 2740, 3543, 3579, 3707, 3918, 3936, 4005, 4015, 4016, 4023, 4026, 4032, 4051, 460101-4605, 4607N, 4608, 4610, 4614-4616, 4805, 4808, 4815, 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, 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, 180101-1813, 1817N, 1818, 1820, 1823N-1829, 2701-2703, 270502-270510, 2707-2709, 2711-2714, 2716, 2717, 2723, 2726-2729, 2731-2739, 2741-2743, 3531, 3560, 3587, 3702, 3709, 3712, 3714, 380101-3809, 3811-3817, 39010101-39010102L, 390103-3917, 391901-3920, 3922-3934, 3937-3941, 3943-3945												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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165	165	155	145	370	140	95	530	440	Length of spec.
									PCR product
13	14	15	16	17	18	19	20	21	Well No.
	14								*070201-0708, 0710, 0712-0716, 071801-0724, 0726-0747, 0749N, 0750, 0752-0757, 0759-0766, 0806, 0820, 15010101-150104, 150106-150108, 1503, 1504, 1506, 1508-1512, 1514, 1515, 1518, 1519, 1523, 1524, 1526N-1530, 1532-1535, 1537-1540, 1543, 1545-1547, 1549, 1550, 1553, 1554, 1556-1558, 1560, 1561, 1563-1566, 1569-1574, 1576, 1578, 1579N, 1581, 1582, 1587, 1590, 1592-1594N, 1596-1599, 9501, 9503, 9504, 9508-9510, 9513, 9515-9520, 9522, 9525, 9527-9529, 9531-9535, 9537, 9538, 9540-9543, 9546-9549N, 1819, 1821, 270401-270402, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2725, 2740, 3543, 3579, 3707, 3918, 3936, 4005, 4015, 4016, 4023, 4026, 4032, 4051, 460101-4605, 4607N, 4608, 4610, 4614-4616, 4805, 4808, 4815, 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, 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, 180101-1813, 1817N, 1818, 1820, 1823N-1829, 2701-2703, 270502-270510, 2707-2709, 2711-2714, 2716, 2717, 2723, 2726-2729, 2731-2739, 2741-2743, 3531, 3560, 3587, 3702, 3709, 3712, 3714, 380101-3809, 3811-3817, 39010101-39010102L, 390103-3917, 391901-3920, 3922-3934, 3937-3941, 3943-3945
13	14	15	16	17	18	19	20	21	Well No.

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Length of spec.	90	150	240	190	90	170	80	250	155	270	205	235
PCR product				230								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
400101-400203, 4004, 40060101-401403, 4018-4022N, 4024, 4025, 4027, 4029-4031, 4033-4040, 4042-4050, 4052-4076, 4078-4086, 4088-4092, 4101-4108, 4201, 4202, 4204-4208, 4415, 4418, 4420, 4431, 4441, 4447, 4453, 4454, 4501-4509, 4606, 4609, 4611, 4613, 47010101-4705, 480101-480102, 480301-4804, 4806, 4807, 4809-4813, 4816-4819, 5401-5405N, 5407-5415, 550201-550202, 5504, 5507, 5508, 5510, 5512, 5513, 5516, 5518-5520, 5522, 5523, 5526, 5527, 5530, 5532, 5601, 5602, 5604, 5607, 5608, 5610, 5613-5620, 5622, 5623, 5901-5903, 670101-6702, 8101, 8102, 8104N, 8201, 8202												
*0758, 9502						6						
*0828, 0835, 1555, 1814, 4003, 4209												
*1301, 1306, 1307N, 1312, 1313, 1317, 1322, 1588, 2719, 2730, 3546, 3557, 370101-370104, 3703N, 3705, 3706, 3708, 3710, 3711, 3942, 44020101-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-446402, 5609, 5611, 5612, 570101-5708, 5710, 5712, 5715-5717, 8301		2										
*1316, 1401-140202, 1404-1409, 1542, 1815, 3810, 3935, 4901-4905, 5001, 5002, 5004, 5207, 550101-550104, 5503, 5505, 5511, 5515, 5517, 5524, 5525, 5528, 5529, 5531, 560501-5606						6	7					
*1320, 5514		2				6	7					
*1403, 5509						6	w					
*1502, 1513, 1521, 1525, 1536, 1544, 1562, 1577, 1580, 1585, 1589, 9506, 9512, 9521, 9539, 9544, 3713, 5709		2				6						
*1516-151702, 1567, 1595						6						
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **13F**

Lot-specific Information

165	165	155	145	370	140	95	530	440	Length of spec.
									PCR product
13	14	15	16	17	18	19	20	21	Well No.
14									400101-400203, 4004, 40060101-401403, 4018-4022N, 4024, 4025, 4027, 4029-4031, 4033-4040, 4042-4050, 4052-4076, 4078-4086, 4088-4092, 4101-4108, 4201, 4202, 4204-4208, 4415, 4418, 4420, 4431, 4441, 4447, 4453, 4454, 4501-4509, 4606, 4609, 4611, 4613, 47010101-4705, 480101-480102, 480301-4804, 4806, 4807, 4809-4813, 4816-4819, 5401-5405N, 5407-5415, 550201-550202, 5504, 5507, 5508, 5510, 5512, 5513, 5516, 5518-5520, 5522, 5523, 5526, 5527, 5530, 5532, 5601, 5602, 5604, 5607, 5608, 5610, 5613-5620, 5622, 5623, 5901-5903, 670101-6702, 8101, 8102, 8104N, 8201, 8202
									*0758, 9502
14				19					*0828, 0835, 1555, 1814, 4003, 4209
	14								*1301, 1306, 1307N, 1312, 1313, 1317, 1322, 1588, 2719, 2730, 3546, 3557, 370101 370104, 3703N, 3705, 3706, 3708, 3710, 3711, 3942, 44020101-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-446402, 5609, 5611, 5612, 570101-5708, 5710, 5712, 5715-5717, 8301
14									*1316, 1401-140202, 1404-1409, 1542, 1815, 3810, 3935, 4901-4905, 5001, 5002, 5004, 5207, 550101-550104, 5503, 5505, 5511, 5515, 5517, 5524, 5525, 5528, 5529, 5531, 560501-5606
	14								*1320, 5514
14									*1403, 5509
	14								*1502, 1513, 1521, 1525, 1536, 1544, 1562, 1577, 1580, 1585, 1589, 9506, 9512, 9521, 9539, 9544, 3713, 5709
14				20					*1516-151702, 1567, 1595
13	14	15	16	17	18	19	20	21	Well No.

Lot No.: **13F**

Lot-specific Information

Length of spec.	90	150	240	190	90	170	80	250	155	270	205	235
PCR product				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, 3533-3536, 3538-3542, 3545, 3547-3550, 3552-3556, 3559, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3580, 3582-3585, 3590-3594, 4802, 5142, 530101-530104, 5303-5305, 5309-5313, 5315, 5316		2										
*9524, 4077, 4087					5							
*1822, 3704, 5714		2								10		
*3505, 3551, 3566, 3572, 5314												
*350901-350902, 3518, 3522, 3532, 3537, 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		
*3544, 3567, 3586, 512402, 5136						6						
*3558						6	7					
*4028		2				6	7			10		
*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, , 520101-5202, 5204-520602, 5208, 5209, 5211-5214, 5621, 7801-7803, 7805, 7806						6	7					
*5129, 5155						6	w					
*5153							7					
*5711			3								11	
HLA-B allele ⁴												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Cw*0317			3									
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **13F**

Lot-specific Information

165	165	155	145	370	140	95	530	440	Length of spec.
									PCR product
13	14	15	16	17	18	19	20	21	Well No.
13									*1520, 350101-350403, 3506-350803, 3510, 3512, 3513, 3515-3517, 3519-352002, 3523, 3525-3530, 3533-3536, 3538-3542, 3545, 3547-3550, 3552-3556, 3559, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3580, 3582-3585, 3590-3594, 4802, 5142, 530101-530104, 5303-5305, 5309-5313, 5315, 5316
	14								*9524, 4077, 4087
	14								*1822, 3704, 5714
13						19			*3505, 3551, 3566, 3572, 5314
13									*350901-350902, 3518, 3522, 3532, 3537, 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									*3544, 3567, 3586, 512402, 5136
13						19			*3558
	14								*4028
	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, , 520101-5202, 5204-520602, 5208, 5209, 5211-5214, 5621, 7801 7803, 7805, 7806
13									*5129, 5155
13									*5153
	14								*5711
									HLA-B allele ⁴
13	14	15	16	17	18	19	20	21	Well No.
									Cw*0317
13	14	15	16	17	18	19	20	21	Well No.

Lot No.: 13F

Lot-specific Information

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

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

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

³The nucleotide position, in the 2nd, 3rd or 4th exon or the 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.

⁴The B*5803 allele was never assigned.

⁵Primer 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.

Lot No.: **13F**

Lot-specific Information

CELL LINE VALIDATION SHEET																						
HLA-B*58 SSP subtyping kit																						
					Well																	
						1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Prod. No.:	200851501	200618102	200618103	200738004	200618105	200618106	200851507	200618108	200851509	200618110	200618111	200618112	200618113	200618114	200624685	200624716		
	IHWC 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	9007	DEM	*5701		-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
9	9026	YAR	*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
10	9107	LKT3	*5401		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
11	9051	PITOUT	*440301		-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
12	9052	DBB	*5701		-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	
13	9067	BTB	*270502		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	
14	9071	OLGA	*1501	*1520	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	
15	9075	DKB	*4001		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
16	9037	SWEIG007	*4002		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
17	9008	WILJON	*1801		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
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	+	+	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	

Lot No.: **13F**

Lot-specific Information

CELL LINE VALIDATION SHEET										
HLA-B*58 SSP subtyping kit										
					Well					
						17	18	19	20	21
				Prod. No.:	200851517	200851518	200851519	200851520	200851521	
	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	9007	DEM	*5701		-	-	-	-	-	
9	9026	YAR	*3801		-	-	-	-	-	
10	9107	LKT3	*5401		-	-	-	-	-	
11	9051	PITOUT	*440301		-	-	-	-	-	
12	9052	DBB	*5701		-	-	-	-	-	
13	9067	BTB	*270502		-	-	-	-	-	
14	9071	OLGA	*1501	*1520	-	-	-	-	-	
15	9075	DKB	*4001		-	-	-	-	-	
16	9037	SWEIG007	*4002		-	-	-	-	-	
17	9008	WILJON	*1801		-	-	-	-	-	
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	-	-	-	-	-	

Lot No.: **13F**

Lot-specific Information

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-B*58 SSP

Product number: 101.568-06u – without *Taq* polymerase
Lot number: 13F
Expiry date: 2010-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	2008-515-09	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	2008-515-20
5	2006-181-05	13	2006-181-13	21	2008-515-21
6	2006-181-06	14	2006-181-14		
7	2008-515-07	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 and 20 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 and 20 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-May-27

Approved by:

Quality Control, Supervisor

Lot No.: **13F**

Lot-specific Information

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*58
Product number: 101.568-06u
Lot number: 13F

Intended use: HLA-B*58 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-May-27

Olle Olerup
Managing Director

Lot No.: **13F**

Lot-specific Information

Lot No.: **13F**

Lot-specific Information

Lot No.: **13F**

Lot-specific Information

Lot No.: **13F**

Lot-specific Information

ADDRESSES:

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