

Lot No.: **35G**

Lot-specific Information

***Olerup SSP*[®] HLA-B*58**

Product number: 101.568-06u – without *Taq* 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*[®] 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. 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.

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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*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 2009¹ 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 2009-July-17, release 2.26.0, www.ebi.ac.uk/imgt/hla.

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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

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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
1 ⁴	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, Cw*0317
4 ⁸	190 bp, 230 bp	1070 bp	*5804, 5815	
5 ⁴	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
7^{4,5}	80 bp	1070 bp	*5806, 5808, 5819	*1316, 1320, 1401-140202, 1403 ^w , 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 ^w , 5130, 5132-5135, 5137-5139, 5141N, 5143, 5145, 5146, 5148-5153, 5155 ^w , 5156-5160, 5162-5165, 520101-5202, 5204-5209, 5211-5214, 5306, 5308, 550101-550104, 5503, 5505, 5509 ^w , 5511, 5514, 5515, 5517, 5524, 5525, 5528, 5529, 5531, 5533, 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	*1410, 1822, 3521, 3524, 3581, 3704, 4028, 5104, 5146, 5156, 5302, 5306, 5714

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11	205 bp	1070 bp	*5802, 5807	5711, A*2464^W, Cw*0106^W
12	235 bp	1070 bp	*5810N	
13⁹	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 [?]
14^{6,9}	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 [?]
15	155 bp	1070 bp	*5812	
16	145 bp	1070 bp	*5813	
17⁷	375 bp	1070 bp	*5816, 5818	
18	140 bp	1070 bp	*5817N	
19⁴	95 bp	800 bp	*5818	*0709, 0711, 0717, 0828, 0835, 0837, 1507, 1555, 1568, 9526, 1814, 3505, 3551, 3558, 3566, 3572, 3589, 3597, 4003, 4209, 4612, 4814, 5314
20	170 bp	1070 bp	*5821	
21	440 bp	1070 bp	*5814	

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¹ 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.

² 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.

³ 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.

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

⁵ Primer mix 7 may give rise to unspecific amplifications.

⁶ Primer mix 14 has a tendency of primer dimer formation.

⁷ Primer mixes 17 has a tendency of giving rise to nonspecific amplifications.

⁸ 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.

⁹ 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.

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INTERPRETATION TABLE												
HLA-B*58 SSP subtyping												
Amplification patterns of the B*5801 to B*5821 alleles												
	Well⁵											
	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. control¹												
5'-primer(s)²	209 5'-ggA ³	355 5'-TCA ³	361 5'-AgT ³	106 5'-CCA ³	463 5'-TgA ³	527 5'-TgA ³	486 5'-ACC ³	361 5'-AgT ³	141 5'-ATT ³	355 5'-TCA ³	361 5'-AgT ³	367 5'-T.T ³
3'-primer(s)³	256 5'-CCC ³	463 5'-gCT ³	559 5'-CAg ³	256 5'-CTC ³	512 5'-CCA ³	3 rd 5'-TAT ³	527 5'-CCT ³	572 5'-gCg ³	256 5'-CCC ³	583 5'-gTg ³	527 5'-CCA ³	559 5'-CAg ³
				296 5'-CTg ³								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-B allele⁴												
*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

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INTERPRETATION TABLE									
HLA-B*58 SSP subtyping									
Amplification patterns of the B*5801 to B*5821 alleles									
Well⁵									
13	14	15	16	17	18	19	20	21	
165	165	155	145	375	140	95	170	440	Length of spec. PCR product(s)
1070	1070	1070	1070	1070	1070	800	1070	1070	Length of int. pos. control¹
652	652	142	454	281	209	363	209	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'-ggA ³	5'-gAC ³	
774	774	256	559	369	309	419	340	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'-ggT ³	5'-TCA ³	
13	14	15	16	17	18	19	20	21	Well No.
									HLA-B allele⁴
13									*580101, 580102
13									*5802
13									*5804
13									*5805
13									*5806
13									*5807
13									*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									*5819
	14								*5820
13							20		*5821
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(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.

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Lot-specific Information

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

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
	14								*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

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 ⁴												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **35G**

Lot-specific Information

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 ⁴
13	14	15	16	17	18	19	20	21	Well No.

Lot No.: **35G**

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.: **35G**

Lot-specific Information

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	+	+	+	-	-	-	-	-	-	-	+	-	+	-	-	-

Lot No.: **35G**

Lot-specific Information

CELL LINE VALIDATION SHEET									
HLA-B*58 SSP subtyping kit									
				Prod. No.:	Well				
					17	18	19	20	21
				200851517					
				200851518					
				200851519					
				200964322					
				200851521					
	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	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 OLG A	*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		-	-	-	-	-

Lot No.: **35G**

Lot-specific Information

CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-B*58 SSP

Product number: 101.568-06u – without *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

Lot No.: **35G**

Lot-specific Information

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*58
Product number: 101.568-06u
Lot number: 35G

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 9001:2008 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.

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Saltsjöbaden, Sweden
2009-October-23

Olle Olerup
Managing Director

Lot No.: **35G**

Lot-specific Information

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