

Olerup SSP[®] HLA-B*53

Product number:	101.549-06 – including <i>Taq</i> polymerase
Lot number:	56G
Expiry date:	2011-December-01
Number of tests:	6
Number of wells per test:	16
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. 56G.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP[®] HLA-B*53 LOT.

The HLA-B*53 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP[®]* HLA-B*53 lot was made (**Lot No. 08F**).

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

Well	5'-primer	3'-primer	rationale
3	-	Added	Primer from well 5.
4	Added	Added	Primer pair for the B*5318 allele.
5	Modified	Moved	Primer moved to well 3 and modified 5'-primer for increased specificity.
7	Modified	-	Modified 5'-primer for improved specificity.
9	Modified	Modified	Modified primer pair for improved specificity.
14	-	-	Exchanged positive control primer pair.
15	Added	Added	Primer pair for the B*5319 allele, exchanged positive control primer pair.

PRODUCT DESCRIPTION

HLA-B*53 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*5301 to B*5319 alleles.

PLATE LAYOUT

Each HLA-B*53 test consists of 16 PCR reactions in a 16 well cut PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16

The 16 well PCR plate is marked with 'HLA-B*53' in silver/gray ink.

Well No. 1 is marked with the Lot No. '56G'.

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 16 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*53 SSP subtypings will be influenced by the B*07, eight B*08, the B*13, the B*14, most B*15, the B*18, the B*27, the B*35, the B*37, the B*38, the B*39, the B*40, three B*42, the B*44, the B*45, the B*46, the B*47, the B*48, the B*49, the B*50, the B*51, the B*52, the B*54, the B*55, the B*56, the B*57, the B*58, the B*59, the B*67, the B*78, the B*81, the B*82 and the B*8301 alleles when present on the other haplotype. In addition, the A*9288 allele will be weakly amplified by primer mix 10, the Cw*0312, Cw*0319 and Cw*0326 alleles will be weakly amplified by primer mix 4, the Cw*0336 allele will be amplified by primer mix 9, the Cw*0408, Cw*0527 and Cw*1515 alleles will be amplified by primer mix 4, the Cw*0434 will be amplified by primer mixes 4 and 9, the Cw*0620 and Cw*0781 alleles will be weakly amplified by primer mix 11 and the Cw*0808 allele will be amplified by primer mix 14.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*53, i.e. **B*5301 to B*5319**, recognized by the HLA Nomenclature Committee in October 2009¹ will be amplified by the primers in the HLA-B*53 SSP kit.

The HLA-B*53 primer set cannot distinguish the B*530101 to B*530105 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2009-October-19, release 2.27.0, www.ebi.ac.uk/imgt/hla.

²The B*5319, B*5203 and B*5210 give rise to identical amplification patterns with the HLA-B*53 subtyping kit. These three alleles can be distinguished by the HLA-B low resolution kit and/or the HLA-B*52 subtyping kit.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The nineteen HLA-B*53 alleles can be combined in 190 homozygous and heterozygous combinations. 147 of these genotypes do not give rise to unique amplification patterns. (The different lengths of the specific PCR products in lanes 3, 6, 14 and 15 were not considered in these calculations.)

+++-----+ 5303, 5306 = 5305, 5306 = 5306, 5315 = 5306, 5316
+++-----+ 5302, 5303 = 5302, 5305 = 5302, 5315 = 5302, 5316
++-+-----+ 5302, 5304 = 5302, 5318
++-----+ 5306, 5311 = 5306, 5312 = 5306, 5313
++-----+ 5302, 5311 = 5302, 5312 = 5302, 5313
++-----+ 5301, 5306 = 5302, 5306 = 5302, 5308
++-----+ --+-----+ 5306, 5306 = 5306, 5308
++-----+ +++-----+ 5302, 5317 = 5302, 5319
++-----+ +++-----+ 5301, 5302 = 5302, 5302
+-+-----+ +++-----+ 5304, 5305 = 5305, 5318
+-+-----+ +++-----+ 5303, 5307 = 5307, 5315 = 5307, 5316
+-+-----+ +++-----+ 5303, 5304 = 5303, 5318 = 5304, 5315 = 5304, 5316 =
5315, 5318 = 5316, 5318
+-+-----+ +++-----+ 5305, 5311 = 5305, 5312 = 5305, 5313 = 5312, 5315 =
5312, 5316
+-+-----+ +++-----+ 5309, 5315 = 5309, 5316
+-+-----+ +++-----+ 5311, 5315 = 5313, 5315 = 5313, 5316
+-+-----+ +++-----+ 5305, 5317 = 5305, 5319
+-+-----+ +++-----+ 5301, 5305 = 5303, 5305 = 5305, 5305 = 5305, 5315 =
5305, 5316
+-+-----+ +++-----+ 5303, 5310 = 5310, 5315 = 5310, 5316
+-+-----+ +++-----+ 5303, 5308 = 5308, 5315 = 5308, 5316
+-+-----+ +++-----+ 5303, 5314 = 5314, 5315 = 5314, 5316
+-+-----+ +++-----+ 5303, 5317 = 5303, 5319 = 5315, 5317 = 5315, 5319 =
5316, 5317 = 5316, 5319
+-+-----+ +++-----+ 5301, 5303 = 5301, 5315 = 5301, 5316 = 5303, 5315 =
5303, 5316 = 5315, 5315 = 5315, 5316
+---+-----+ +++-----+ 5304, 5312 = 5312, 5318
+---+-----+ +++-----+ 5307, 5311 = 5307, 5313
+---+-----+ +++-----+ 5304, 5309 = 5309, 5318
+---+-----+ +++-----+ 5304, 5311 = 5304, 5313 = 5311, 5318 = 5313, 5318
+---+-----+ +++-----+ 5304, 5310 = 5307, 5310 = 5307, 5314 = 5310, 5318
+---+-----+ +++-----+ 5301, 5307 = 5307, 5318
+---+-----+ +-+-----+ 5304, 5307 = 5307, 5307
+---+-----+ +++-----+ 5304, 5314 = 5314, 5318
+---+-----+ +++-----+ 5304, 5317 = 5317, 5318 = 5318, 5319
+---+-----+ +++-----+ 5301, 5304 = 5301, 5318 = 5304, 5318 = 5318, 5318
+---+-----+ +++-----+ 5312, 5317 = 5312, 5319
+---+-----+ +++-----+ 5310, 5311 = 5310, 5313
+---+-----+ +++-----+ 5308, 5311 = 5308, 5313
+---+-----+ +++-----+ 5311, 5314 = 5313, 5314
+---+-----+ +++-----+ 5301, 5309 = 5309, 5317 = 5309, 5319 = 5311, 5317 =
5311, 5319 = 5313, 5317 = 5313, 5319
+---+-----+ +++-----+ 5301, 5311 = 5301, 5313
+-----+-+ +++-----+ 5310, 5317 = 5310, 5319
+-----+-+ +++-----+ 5301, 5310 = 5310, 5310 = 5310, 5314
+-----+-+ +++-----+ 5314, 5317 = 5314, 5319
+-----+-+ +++-----+ 5301, 5314 = 5314, 5314
+-----+-+ +++-----+ 5301, 5317 = 5301, 5319 = 5317, 5317 = 5317, 5319

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--+----- +++-----+5303, 5311 = 5303, 5313
-----+--- +++-----+5311, 5312 = 5312, 5312 = 5312, 5313
-----+--- +++-----+5309, 5309 = 5309, 5311 = 5309, 5313
-----+--- +++-----+5311, 5313 = 5313, 5313

5301= 530101-530105

SPECIFICITY TABLE

HLA-B*53 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-B*53 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*53 alleles	Other amplified HLA Class I alleles ³
1	145 bp	800 bp	*530101-5302, 5304-5308, 5310, 5314-5319	*3806, 3807, 4406, 4903, 510101-512402, 5126-5146, 5148, 5149, 5151-5153, 5155-5177, 5179-5184, 520101-520105, 5203-5215, 580101-5802, 5804-5816, 5818-5824
2	270 bp	1070 bp	*5302, 5306	*1410, 1822, 3521, 3524, 3581, 3596, 3704, 4028, 5104, 5146, 5156, 5714, 5809
3 ⁸	135 bp, 290 bp	800 bp	*5303, 5305, 5315, 5316	*2752, 370101-370105, 370107-3705, 3707-3709, 3712, 3713, 3715-3719, 4213, 5209
4 ⁵	190 bp	800 bp	*5304, 5307, 5318	*1303, 1558, 1573, 9533, 9537, 350301, 350302, 3506, 3513, 3536, 3538, 3555, 3556, 3559, 3570, 3574, 3575, 3584, 3585, 3598, 3932, 3933, 4071, 4410, 4415, 4418, 4501, 4502, 4504-4507, 4611, 490101-4903, 4906, 500101-5002, 5004-5009, 511301, 511302, 5115, 5162, 5214, 5403, 560101-5602, 5604, 5607, 5608, 5611, 5613, 5614, 5616, 5617, 5620, 5622, 5624-5626, 5904, 8201, 8202, Cw*0312^w, Cw*0319^w, Cw*0326^w, Cw*0408, Cw*0434, Cw*0527, Cw*1515
5	145 bp	800 bp	*5309, 5311-5313	*1809, 3710, 4483, 5154, 5178
6 ⁹	205 bp, 235 bp	800 bp	*5302, 5305, 5306, 5312	*1325, 153801, 153802, 9515, 180101-1809, 1812-1815, 1817N-1820, 1822-1825, 1827-1834, 1836-1840, 1842-1845, 3521, 3524, 3943, 4415, 5137, 5145, 5163, 5714, 5809
7 ^{4,5}	130 bp	800 bp	*5307, 5310	*1532, 1818, 370101-370107, 3703N-3706, 3708, 3710, 3711, 3713-3718, 3909

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8⁵	170 bp	1070 bp	*5306, 5308	*070201-0724, 0726-0747, 0749N, 0750, 0752-0799, 0806, 0820, 1316, 1320, 1401-1416, 15010101-150104, 150106-1504, 1506-1519, 1521, 1523- 1530, 1532-1540, 1542-1547, 1549, 1550, 1553, 1554, 1556-1558, 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, 9546-9550, 9552-9554, 9556-9561, 9563-9575, 9577, 9578, 1815, 1819, 1821, 1830, 270401-270403, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2725, 2740, 2754, 3511, 351401, 351402, 3521, 3543, 3544, 3558, 3567, 3579, 3586, 3596, 3599, 3707, 3713, 3810, 3918, 3935, 3936, 4005, 4015, 4016, 4023, 4026, 4028, 4032, 4051, 4095, 4098, 4476, 4479, 460101-4605, 4607N, 4608, 4610, 4612, 4614-4617, 4620, 4622, 4805, 4808, 4815, 490101-4907, 500101- 5002, 5004-5009, 510101-5104, 5106, 5107, 5111N-5114, 5116-5118, 5121- 512402, 5126-5130, 5132-5139, 5141N, 5143, 5145, 5146, 5148-5152, 5155-5172, 5174-5180, 5182-5184, 520101-5202, 5204-5209, 5211-5216, 5406, 550101-550106, 5503, 5505, 5509, 5511, 5514, 5515, 5517, 5521, 5524, 5525, 5528, 5529, 5531, 5533, 5536, 5538, 5603, 560501-5606, 5621, 5625, 5709, 5713, 5722, 5806, 5808, 5819, 7801-7803, 7805, 7806
9^{4,6}	75 bp	1070 bp	*530101- 5305, 5307, 5309-5319	*0821, 0825, 1303, 1304, 1505, 1520, 1531, 1548, 1551, 1555, 1584, 1591, 9507, 9523, 9536, 9551, 9555, 1836, 2738, 2747, 350101-3510, 351201- 3513, 3516-352002, 3522-3530, 3532, 3534, 3536-3542, 3545-3557, 3559, 3561-3564, 356801-3572, 3574-3578, 3580-3585, 3589-3592, 3594, 3595, 3597, 3598, 3932, 3933, 4063, 4071, 4092, 44020101-440207, 440208 ^w , 440209-440308, 4405-4415, 4417- 4420, 4422-4429, 4432-4455, 4457-

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				4463, 4465-4475, 4477, 4478, 4480-4488, 4501-4507, 4509, 4609, 4611, 461301, 461302, 4619, 4802, 5105, 5108-510902, 5115, 5119, 5120, 5140, 5142, 5144N, 5154, 5173, 5203, 5210, 5403, 5508, 560101-5602, 5604, 5607-5609, 5611, 5613-5617, 5620, 5622, 5624, 5626, 570101, 570102, 570104-5708, 5710-5712, 5714-5721, 5723, 5724, 580101-5802, 5804, 5807, 5809-5818, 5821-5824, 5904, 7804, 8201, 8202, 8301, Cw*0336, Cw*0434
10	150 bp	1070 bp	*530101-5303, 5305, 5309, 5310, 5311 ^w , 5312-5315, 5316 ^w , 5317, 5318	1304, 1310, 1326, 1505, 1520, 1531, 1552, 1555, 1584 ^w , 1591, 9507, 9514, 9523, 9524, 9536, 9551, 9555, 180101-1812, 1813 ^w , 1814, 1817N, 1818, 1820, 1822-1825, 1827-1829, 1831-1840, 1842-1845, 2741, 350101-350113, 3505, 3507, 350801 ^w -350804 ^w , 3510, 3515-3517, 3519-352002, 3523-3530, 3532, 3535, 3537, 3540N-3542, 3545 ^w , 3546-3554, 3557, 3561 ^w , 3562 ^w , 3563, 3564, 3566 ^w , 356801-3569, 3571, 3572, 3576-3578, 3580 ^w , 3582, 3589-3594, 3597, 3907, 3943, 4003, 4020, 4038, 4052, 4059, 4060, 4443, 4606, 461301, 461302, 4619, 4621, 4802, 4814, 5409, 5414, 5523, 5532, 5609, 5618, 570101-570105, 5706, 5708, 5710, 5711, 5714-5716, 5718, 5719 ^w , 5720, 5721, 5723, 5725, 5726, 580101-5802, 5804, 5807, 5809-5818, 5821, 5822 ^w , 5823, 5824, A*9288^w

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11 ^{4,6}	85 bp	800 bp	*530101-5303, 5305, 5306, 5308-5318	*1304, 1310 ^w , 1326, 15010101-150104, 150106-150112, 150201 ^w , 150202 ^w , 150301-1505, 1506 ^w , 1507, 1508, 151101-1512, 1513 ^w , 1514-1516, 151801-1520, 1521 ^w , 1523, 1524, 152501 ^w , 152502 ^w , 1526N-1529, 1531 ^w , 1532, 1534, 1535, 1536 ^w , 153801-1540, 1543, 1544 ^w , 1546, 1547, 1549-1554, 1555 ^w , 1556, 1557, 1560-1562, 1564-1571, 1574-1576, 1579N-1582, 1584, 1585, 1587, 1588 ^w , 1589 ^w , 1591-1598, 9501-9505, 9506 ^w , 9508-9511N, 9512 ^w , 9513-9515, 9517-9520, 9521 ^w , 9522-9527, 9528 ^w , 9529, 9531, 9532, 9534-9536, 9538, 9539 ^w , 9540, 9542, 9543, 9544 ^w , 9545-9549N, 9551-9555, 9556 ^w , 9557-9561, 9563-9567, 9569, 9570 ^w , 9571-9576, 9578, 180101-180103, 180105-1815, 1817N-1819, 1820 ^w , 1822-1825, 1827-1839, 1842-1845, 2741, 350101-350113, 3505, 3507-350804, 3510, 3511, 351401-3517, 3519-3521, 3523-3530, 3532, 3535, 3537, 3540N-3543, 3545-3552, 3554, 3557, 3558, 3561-3563, 3566-3569, 3571, 3572, 3576-3580, 3582, 3586, 3589-3594, 3597, 3599, 3907, 3943, 4417, 4443, 4509, 460101-4605, 4607N-4610, 4612-4617, 4619-4622, 4802, 4814, 4904, 4905 ^w , 5137, 5145, 5163, 5406, 5409, 5414, 5514, 5523, 5532, 5603, 5609, 5618, 570101-570104, 5706, 5708, 5710, 5711, 5714-5716, 5718-5726, 580101, 580102, 5802, 5804-5807, 5809-5819, 5821-5824, Cw*0620^w , Cw*0781^w
12 ⁴	105 bp	1070 bp	*0778, 130201-1303, 1308Q, 1309, 1314-1316, 1318, 1319, 1327, 1330, 1542, 3560, 4415, 4418, 4501, 4503-4508, 4510, 4611, 4618, 490101-4903, 4906, 4907, 500101-5002, 5004-5008, 5115, 5401-5403, 5405N, 5407, 5408N, 5410, 5412, 5413, 5416-5419, 550101-550103, 550105-5503, 5505, 5507, 5509-5512, 5515, 5516, 5518, 5519, 5521, 5522, 5524-5526, 5529-5531, 5533-5538, 560101, 560102,	

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				5607, 5608, 5613, 5614, 5616, 5617, 5619N, 5620, 5623-5626, 5901, 5904, 5905
13⁴	90 bp	1070 bp		*580101-5802, 5804-5824
14^{4,6,10}	95 bp, 130 bp	1070 bp	*5310, 5314	*0709, 0711, 0717, 0828, 0835, 0837, 1405, 1507, 1555, 1568, 1814, 3505, 3551, 3558, 3566, 3572, 3819, 3903, 3924, 3937, 4003, 4206, 4209, 4612, 4807, 4814, Cw*0808
15^{4,7,11}	105 bp, 205 bp	800 bp	*5309, 5317, 5319	*180101-1806, 1808-1815, 1817N-1828, 1830-1832, 1834-1836, 1838-1845, 350201-350203, 352001, 352002, 3528, 3572, 3581, 3583, 3595, 370101-370105, 370107-3705, 3707-3719, 4483, 4903, 5107, 520101-520105, 5203-5205, 5207-5215, 7805, 7806
16	160 bp	1070 bp	*530101-5310, 5312-5315, 5317-5319	*0726, 0734, 0743, 0748, 0780, 0813, 0825, 130101-1304, 1306-1317, 1319-1323, 1325-1330, 1401-140203, 1404-1416, 150201-150303, 1505, 1506, 1509, 1510, 1513, 1516-151803, 1520, 1521, 1523, 152501, 152502, 1529, 1531, 1536, 1537, 1539, 1540, 1542, 1544, 1548, 1552, 1555, 1561, 1562, 1564, 1567, 1569, 1572, 1574, 1580, 1586, 1588 ^w , 1589-1591, 1593, 1595, 1598, 9503, 9506-9508, 9512, 9514, 9515, 9519, 9521, 9523, 9524, 9527, 9531-9534, 9536, 9538, 9539, 9551, 9553, 9555, 9556, 9558, 9561, 9562, 9570, 9573, 9576 ^w , 9577, 180101-1812, 1814, 1815, 1817N, 1818, 1820, 1822-1829, 1831-1845, 2701-2721, 2723, 2724, 2726-2744, 2746, 2748-2759N, 350101-3507, 350901-3513, 3515-3517, 3519-3537, 3539-3542, 3546-3560, 3563-3565Q, 356801-3572, 3574-3578, 3581-3585, 3588-3598, 3702, 380101-3820, 39010101, 39010102L, 390103-3907, 3909, 3910, 3912-3917, 391901-3920, 3922-3932, 3934, 3935, 3937-3954, 400101-401403, 4018-4022N, 4024-4031, 4033-4040, 4042-4097, 440301-440308, 4407, 4410, 4413, 4426, 4429-4432, 4436-4440, 4443, 4445-4447, 4450, 4454, 4457, 4460, 4461N,

446401-4465, 4469, 4477, 4479, 4481, 4482, 4485, 4606, 4608, 4611, 461301, 461302, 4618, 4619, 4621, 47010101-4705, 480101-4804, 4806, 4807, 4809-4818, 4820, 490101, 490102^w, 4902-4907, 500101-5002, 5004-5009, 510101-5104, 5106, 5107, 510901-5119, 5121-512402, 5126-5128, 5130-5135, 5137-5143, 5145, 5146, 5148-5152, 5153^w, 5156-5160, 5162-5171, 5172^w, 5173-5181, 5183, 5184, 520101-5216, 5401-5405N, 5407-5409, 5411-5419, 550101-5505, 5507, 5508, 5510-5519, 5522-5536, 5538, 560101-5602, 5604-5612, 5614-5626, 570101-570105, 570301, 570302, 5706-5708, 5710, 5711, 5714-5718, 5720-5723, 5725, 5726, 580101-5802, 5804-5821, 5823, 5824, 5901-5905, 670101-6702, 7801-7806, 8101-8104N

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*53 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*53 subtyping.

In addition, wells number 3 to 7, 11 and 15 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 non-HLA-B*53 alleles will be amplified by all 16 primer mixes. In addition, the A*9288 allele will be weakly amplified by primer mix 10, the Cw*0312, Cw*0319 and Cw*0326 alleles will be weakly amplified by primer mix 4, the Cw*0336 allele will be amplified by primer mix 9, the Cw*0408, Cw*0527 and Cw*1515 alleles will be amplified by primer mix 4, the Cw*0434 will be amplified by primer mixes 4 and 9, the Cw*0620 and Cw*0781 alleles will be weakly amplified by primer mix 11 and the Cw*0808 allele will be

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amplified by primer mix 14. The B*5319, B*5203 and B*5210 give rise to identical amplification patterns with the HLA-B*53 subtyping kit. These three alleles can be distinguished by the HLA-B low resolution kit and/or the HLA-B*52 subtyping kit.

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

⁵Primer mix 4, 7 and 8 have a tendency of giving rise to nonspecific amplifications..

⁶Primer mixes 9, 11 and 14 may give a lower yield of specific PCR product than the other HLA-B*53 primer mixes.

⁷Primer mix 15 may have tendencies of primer dimer formation.

⁸Primer mix 3: Specific PCR fragment of 135 bp in the B*5303, 5305 and 5316 and the B*2752, 370101-370105, 370107-3705, 3707-3709, 3712, 3713, 3715-3719 and 5209 alleles. Specific PCR fragment of 290 bp in the B*5315 and the B*4213 alleles.

⁹Primer mix 6: Specific PCR fragment of 205 bp in the B*5302 and 5306 and the B*153801, 153802, 180101-1809, 1812-1815, 1817N-1820, 1822-1825, 1827-1834, 1836-1840, 1842-1845, 3521, 3524, 3943, 5137, 5145, 5163, 5714 and 5809 alleles. Specific PCR fragment of 235 bp in the the B*5305 and 5312 and the B*1325, 9515 and 4415 alleles.

¹⁰Primer mix 14: Specific PCR fragment of 95 bp in the B*5314 and the B*0709, 0711, 0717, 0828, 0835, 0837, 1405, 1507, 1555, 1568, 1814, 3505, 3551, 3558, 3566, 3572, 3819, 3903, 3924, 3937, 4003, 4206, 4209, 4612, 4807 and 4814 and the Cw*0808 alleles. Specific PCR fragment of 130 bp in the B*5310 allele.

¹¹Primer mix 15: Specific PCR fragment of 105 bp in the B*5309 and 5317 and the B*180101-1806, 1808-1815, 1817N-1828, 1830-1832, 1834-1836, 1838-1845, 352001, 352002, 3528, 3572, 3595, 370101-370105, 370107-3705, 3707-3719, 4483, 4903, 5107, 520101-520105, 5203-5205, 5207-5215, 7805 and 7806 alleles. Specific PCR fragment of 205 bp in the B*5319 and the B*350201-350203, 3581, 3583 and 3595 alleles.

‘w’, might be weakly amplified.

INTERPRETATION TABLE

HLA-B*53 SSP subtyping

Amplification patterns of the B*5301 to B*5319 alleles

	Well ⁴							
	1	2	3	4	5	6	7	8
Length of spec.	145	270	135	190	145	205	130	170
PCR product(s)			290			235		
Length of int.	800	1070	800	800	800	800	800	1070
pos. control ¹								
5'-primer(s) ²	206	355	206	49	206	106	106	527
	5' -gAC 3' -TCA 3' -gAC 3' -CAg 3' -gAC 3' -CCA 3' -CCA 3' -TgA 3'							
			355	419		419	368	
	5' -TCA 3' -gTT 3' 5' -gTC 3' -gTC 3'							
3'-primer(s) ³	309	583	301	79	309	302	197	3 rd I
	5' -ATC 3' -gTg 3' -gTC 3' -gTA 3' -gTg 3' -ggC 3' -gAA 3' -TAT 3'							
			302	559		583	463	
	5' -ggC 3' -CAg 3' 5' -gTg 3' -gCT 3'							
			603					
	5' -gTg 3'							
Well No.	1	2	3	4	5	6	7	8
HLA-B allele								
*530101-530105	1							
*5302	1	2				6		
*5303			3					
*5304	1			4				
*5305	1		3			6		
*5306	1	2				6		8
*5307	1			4			7	
*5308	1							8
*5309					5			
*5310	1						7	
*5311					5			
*5312					5	6		
*5313					5			
*5314	1							
*5315	1		3					
*5316	1		3					
*5317	1							
*5318	1			4				
*5319, 5203, 5210 ⁵	1							
Well No.	1	2	3	4	5	6	7	8

INTERPRETATION TABLE								
HLA-B*53 SSP subtyping								
Amplification patterns of the B*5301 to B*5319 alleles								
Well ⁴								
9	10	11	12	13	14	15	16	
75	150	85	105	90	95	105	160	Length of spec. PCR product(s)
					130	205		
1070	1070	800	1070	1070	1070	800	1070	Length of int. pos. control ¹
527	419	419	357	209	106	206	539	5'-primer(s) ²
5'-TgT ^{3'5'}	5'-gTC ^{3'5'}	5'-gTC ^{3'5'}	5'-Tgg ^{3'5'}	5'-ggA ^{3'}	5'-CCA ^{3'5'}	5'-gAC ^{3'5'}	5'-gCT ^{3'}	
					363	397		
					5'-AgC ^{3'5'}	5'-gCT ^{3'}		
559	527	463	420	256	197	272	3 rd I	3'-primer(s) ³
5'-CAg ^{3'}	5'-CCA ^{3'5'}	5'-gCT ^{3'5'}	5'-gCT ^{3'5'}	5'-CTC ^{3'5'}	5'-gAA ^{3'5'}	5'-Tgg ^{3'5'}	5'-TAT ^{3'}	
				256	419	559		
				5'-CCC ^{3'5'}	5'-Cgg ^{3'5'}	5'-CAg ^{3'}		
				419				
				5'-CgA ^{3'}				
9	10	11	12	13	14	15	16	Well No.
								HLA-B allele
							16	*530101-530105
9	10	11					16	*5302
9	10	11					16	*5303
9							16	*5304
9	10	11					16	*5305
		11					16	*5306
9							16	*5307
		11					16	*5308
9	10	11				15	16	*5309
9	10	11			14		16	*5310
9	w	11						*5311
9	10	11					16	*5312
9	10	11					16	*5313
9	10	11			14		16	*5314
9	10	11					16	*5315
9	w	11						*5316
9	10	11				15	16	*5317
9	10	11					16	*5318
9						15	16	*5319, 5203, 5210 ⁵
9	10	11	12	13	14	15	16	Well No.

Lot No.: **56G**

Lot-specific Information

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Length of spec.	145	270	135	190	145	205	130	170
PCR product(s)			290			235		
Well No.	1	2	3	4	5	6	7	8
*070201-0708, 0710, 0712-0716, 071801-0724, 0727-0733, 0735-0742, 0744-0747, 0749N, 0750, 0752-0777, 0779, 0781-0799, 0806, 0820, 1403, 1530, 1533, 1545, 1563, 1577-157802, 1599, 9516, 9541, 9550, 9568, 2725, 3544, 3918, 3936, 4015, 4016, 4023, 4032, 4098, 4476, 4805, 4808, 5709, 5713								8
*0709, 0711, 0717								8
*0726, 0734, 0743, 0780, 1320, 1401-140203, 1404, 140601-1409, 1411-1416, 1509, 1510, 15170101-151702, 1537, 1572, 1590, 9577, 270401-270403, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2740, 2754, 3810, 3935, 4005, 4026, 4051, 4095, 4479, 4815, 5150, 5202, 5216, 550104, 5517, 5528, 560501-5606, 5621, 7801-7803								8
*0748, 0813, 130101, 130102, 1306, 1307N, 1311-1313, 1317, 1321-1323, 1328, 1329, 1586, 9562, 2701-2703, 270502-270512, 2707-2709, 2711-2714, 2716, 2717, 2719, 2723, 2726-2737, 2739, 2742-2744, 2746, 2748-2751, 2753, 2755-2759N, 3531, 3533, 3565Q, 3588, 380101-3805, 3808, 3809, 3811-3818, 3820, 39010101, 39010102L, 390103-390202, 3904-390602, 3910, 3912-3917, 391901-3920, 3922, 3923, 3925N-3931, 3934, 3938Q-3942, 3944-3954, 400101-400207, 4004, 40060101-401403, 4018, 4019, 4021, 4022N, 4024, 4025, 4027, 4029-4031, 4033-4037, 4039, 4040, 4042-4050, 4053-4058, 4061, 4062, 4064-4070, 407201-4091, 4093, 4094, 4096, 4097, 4430, 4431, 446401, 446402, 47010101-4705, 480101, 480102, 480301-4804, 4806, 4809-4813, 4816-4818, 4820, 5404, 5411, 5415, 5504, 5513, 5527, 5610, 5612, 5902, 5903, 670101-6702, 8101-8104N								
Well No.	1	2	3	4	5	6	7	8

Lot No.: **56G**

Lot-specific Information

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75	150	85	105	90	95	105	160	Length of spec. PCR product(s)
9	10	11	12	13	14	15	16	Well No.
								*070201-0708, 0710, 0712-0716, 071801-0724, 0727-0733, 0735-0742, 0744-0747, 0749N, 0750, 0752-0777, 0779, 0781-0799, 0806, 0820, 1403, 1530, 1533, 1545, 1563, 1577-157802, 1599, 9516, 9541, 9550, 9568, 2725, 3544, 3918, 3936, 4015, 4016, 4023, 4032, 4098, 4476, 4805, 4808, 5709, 5713
					14			*0709, 0711, 0717
							16	*0726, 0734, 0743, 0780, 1320, 1401-140203, 1404, 140601-1409, 1411-1416, 1509, 1510, 15170101-151702, 1537, 1572, 1590, 9577, 270401-270403, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2740, 2754, 3810, 3935, 4005, 4026, 4051, 4095, 4479, 4815, 5150, 5202, 5216, 550104, 5517, 5528, 560501-5606, 5621, 7801-7803
							16	*0748, 0813, 130101, 130102, 1306, 1307N, 1311-1313, 1317, 1321-1323, 1328, 1329, 1586, 9562, 2701-2703, 270502-270512, 2707-2709, 2711-2714, 2716, 2717, 2719, 2723, 2726-2737, 2739, 2742-2744, 2746, 2748-2751, 2753, 2755-2759N, 3531, 3533, 3565Q, 3588, 380101-3805, 3808, 3809, 3811-3818, 3820, 39010101, 39010102L, 390103-390202, 3904-390602, 3910, 3912-3917, 391901-3920, 3922, 3923, 3925N-3931, 3934, 3938Q-3942, 3944-3954, 400101-400207, 4004, 40060101-401403, 4018, 4019, 4021, 4022N, 4024, 4025, 4027, 4029-4031, 4033-4037, 4039, 4040, 4042-4050, 4053-4058, 4061, 4062, 4064-4070, 407201-4091, 4093, 4094, 4096, 4097, 4430, 4431, 446401, 446402, 47010101-4705, 480101, 480102, 480301-4804, 4806, 4809-4813, 4816-4818, 4820, 5404, 5411, 5415, 5504, 5513, 5527, 5610, 5612, 5902, 5903, 670101-6702, 8101-8104N
9	10	11	12	13	14	15	16	Well No.

Length of spec.	145	270	135	190	145	205	130	170
PCR product(s)			290			235		
Well No.	1	2	3	4	5	6	7	8
*0778, 5509, 5521								8
*0821, 2747, 3518, 44020101-440207, 440209, 440210, 4405, 4408, 4409, 4411, 4412, 4414, 4419N, 4420, 4422-4425, 4427-442802, 4433-4435, 4441, 4442, 4444, 4448, 4449, 4451-4453, 4455, 4458N, 4459, 4462, 4463, 4466-4468, 4470-4475, 4478, 4480, 4484, 4486-4488, 5702, 5704, 5705, 5712, 8301, <i>Cw</i> *0336								
*0825, 1548, 2738, 350401-350403, 350901, 350902, 351201, 351202, 3522, 3534, 3539, 4063, 4092, 440301-440308, 4407, 4413, 4426, 4429, 4432, 4436-4440, 4445-4447, 4450, 4454, 4457, 4460, 4461N, 4465, 4469, 4477, 4481, 4482, 4485, 5508, 5615, 570301, 570302, 5707, 5717, 7804								
*0828, 0835, 0837, 4206, 4209, <i>Cw</i> *0808								
*130201-130206, 1308Q, 1309, 1314, 1315, 1319, 1327, 1330, 3560, 4618, 5401, 5402, 5405N, 5407, 5408N, 5412, 5413, 5416-5419, 550201-550205, 5507, 5510, 5512, 5516, 5518, 5519, 5522, 5526, 5530, 5534, 5535, 5619N, 5623, 5901, 5905								
*1303, 4611, 5403, 560101, 560102, 5607, 5608, 5614, 5616, 5617, 5620, 5624, 5626, 5904				4				
*1304, 1505, 1520, 1591, 9523, 9536, 9551, 9555, 350101-350113, 3507, 3510, 3516, 3517, 3519, 3523, 3525-3527, 3529, 3530, 3532, 3537, 3540N-3542, 3546-3550, 3552, 3554, 3557, 3563, 356801-3569, 3571, 3576-3578, 3582, 3589-3592, 3594, 3597, 4443, 461301, 461302, 4619, 4802, 5609, 570101, 570102, 570104, 5706, 5708, 5710, 5711, 5715, 5716, 5718, 5720, 5721, 5723								
*1310								
Well No.	1	2	3	4	5	6	7	8

Lot No.: **56G**

Lot-specific Information

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75	150	85	105	90	95	105	160	Length of spec. PCR product(s)
9	10	11	12	13	14	15	16	Well No.
			12					*0778, 5509, 5521
9								*0821, 2747, 3518, 44020101-440207, 440209, 440210, 4405, 4408, 4409, 4411, 4412, 4414, 4419N, 4420, 4422-4425, 4427-442802, 4433-4435, 4441, 4442, 4444, 4448, 4449, 4451-4453, 4455, 4458N, 4459, 4462, 4463, 4466-4468, 4470-4475, 4478, 4480, 4484, 4486-4488, 5702, 5704, 5705, 5712, 8301, Cw*0336
9							16	*0825, 1548, 2738, 350401-350403, 350901, 350902, 351201, 351202, 3522, 3534, 3539, 4063, 4092, 440301-440308, 4407, 4413, 4426, 4429, 4432, 4436-4440, 4445-4447, 4450, 4454, 4457, 4460, 4461N, 4465, 4469, 4477, 4481, 4482, 4485, 5508, 5615, 570301, 570302, 5707, 5717, 7804
					14			*0828, 0835, 0837, 4206, 4209, Cw*0808
			12				16	*130201-130206, 1308Q, 1309, 1314, 1315, 1319, 1327, 1330, 3560, 4618, 5401, 5402, 5405N, 5407, 5408N, 5412, 5413, 5416-5419, 550201-550205, 5507, 5510, 5512, 5516, 5518, 5519, 5522, 5526, 5530, 5534, 5535, 5619N, 5623, 5901, 5905
9			12				16	*1303, 4611, 5403, 560101, 560102, 5607, 5608, 5614, 5616, 5617, 5620, 5624, 5626, 5904
9	10	11					16	*1304, 1505, 1520, 1591, 9523, 9536, 9551, 9555, 350101-350113, 3507, 3510, 3516, 3517, 3519, 3523, 3525-3527, 3529, 3530, 3532, 3537, 3540N-3542, 3546-3550, 3552, 3554, 3557, 3563, 356801-3569, 3571, 3576-3578, 3582, 3589-3592, 3594, 3597, 4443, 461301, 461302, 4619, 4802, 5609, 570101, 570102, 570104, 5706, 5708, 5710, 5711, 5715, 5716, 5718, 5720, 5721, 5723
	10	w					16	*1310
9	10	11	12	13	14	15	16	Well No.

Lot No.: **56G**

Lot-specific Information

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Length of spec.	145	270	135	190	145	205	130	170
PCR product(s)			290			235		
Well No.	1	2	3	4	5	6	7	8
*1316, 1542, 4907, 550101-550103, 550105, 550106, 5503, 5505, 5511, 5515, 5524, 5525, 5529, 5531, 5533, 5536, 5538								8
*1318, 4508, 4510, 5410, 5537								
*1325						6		
*1326, 1552, 9514, 9524, 2741, 3515, 3535, 3593, 3907, 4621, 5409, 5414, 5523, 5532, 5618, 570103, 5725, 5726								
*1405								8
*1410, 3596, 4028		2						8
*15010101-150104, 150106-150112, 1504, 1508, 151101-1512, 1514, 1515, 1519, 1524, 1526N-1528, 1534, 1535, 1543, 1546, 1547, 1549, 1550, 1553, 1554, 1556, 1557, 1560, 1565, 1566, 1570, 1571, 1576, 1579N, 1581, 1582, 1585, 1587, 1592, 1594N, 1596, 1597, 9501, 9502, 9504, 9509, 9510, 9513, 9517, 9518, 9520, 9522, 9525, 9526, 9529, 9535, 9540, 9542, 9543, 9546-9549N, 9552, 9554, 9557, 9559, 9560, 9563-9567, 9569, 9571, 9572, 9574, 9575, 9578, 351401, 351402, 3543, 3567, 3579, 3586, 3599, 460101-4605, 4607N, 4610, 4614-4617, 4620, 4622, 5406, 5603								8
*150201, 150202, 1506, 1513, 1521, 152501, 152502, 1536, 1544, 1589, 9506, 9512, 9521, 9539, 9556, 9570, 4905								8
*150301-150303, 1516, 151801-151803, 1523, 1529, 1539, 1540, 1561, 1562, 1564, 1567, 1569, 1574, 1580, 1593, 1595, 1598, 9503, 9508, 9519, 9527, 9531, 9532, 9534, 9538, 9553, 9558, 9561, 9573, 3511, 4608, 4904, 5514, 5722								8
*1507, 1568, 4612								8
*1531								
*1532							7	8
*153801, 153802						6		8
*1551, 4417, 4509, 4609, 5724								
*1555								
Well No.	1	2	3	4	5	6	7	8

Lot No.: **56G**

Lot-specific Information

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75	150	85	105	90	95	105	160	Length of spec. PCR product(s)
9	10	11	12	13	14	15	16	Well No.
			12				16	*1316, 1542, 4907, 550101-550103, 550105, 550106, 5503, 5505, 5511, 5515, 5524, 5525, 5529, 5531, 5533, 5536, 5538
			12					*1318, 4508, 4510, 5410, 5537
							16	*1325
	10	11					16	*1326, 1552, 9514, 9524, 2741, 3515, 3535, 3593, 3907, 4621, 5409, 5414, 5523, 5532, 5618, 570103, 5725, 5726
					14		16	*1405
							16	*1410, 3596, 4028
		11						*15010101-150104, 150106-150112, 1504, 1508, 151101-1512, 1514, 1515, 1519, 1524, 1526N-1528, 1534, 1535, 1543, 1546, 1547, 1549, 1550, 1553, 1554, 1556, 1557, 1560, 1565, 1566, 1570, 1571, 1576, 1579N, 1581, 1582, 1585, 1587, 1592, 1594N, 1596, 1597, 9501, 9502, 9504, 9509, 9510, 9513, 9517, 9518, 9520, 9522, 9525, 9526, 9529, 9535, 9540, 9542, 9543, 9546-9549N, 9552, 9554, 9557, 9559, 9560, 9563-9567, 9569, 9571, 9572, 9574, 9575, 9578, 351401, 351402, 3543, 3567, 3579, 3586, 3599, 460101-4605, 4607N, 4610, 4614-4617, 4620, 4622, 5406, 5603
		w					16	*150201, 150202, 1506, 1513, 1521, 152501, 152502, 1536, 1544, 1589, 9506, 9512, 9521, 9539, 9556, 9570, 4905
		11					16	*150301-150303, 1516, 151801-151803, 1523, 1529, 1539, 1540, 1561, 1562, 1564, 1567, 1569, 1574, 1580, 1593, 1595, 1598, 9503, 9508, 9519, 9527, 9531, 9532, 9534, 9538, 9553, 9558, 9561, 9573, 3511, 4608, 4904, 5514, 5722
		11			14			*1507, 1568, 4612
9	10	w					16	*1531
		11						*1532
		11						*153801, 153802
9		11						*1551, 4417, 4509, 4609, 5724
9	10	w			14		16	*1555
9	10	11	12	13	14	15	16	Well No.

Length of spec.	145	270	135	190	145	205	130	170
PCR product(s)			290			235		
Well No.	1	2	3	4	5	6	7	8
*1558, 1573, 9537				4				8
*1575, 9505, 9511N, 9545								
*1584, 350801-350804, 3545, 3561, 3562, 3580, 5719								
*1588								
*9507, 3553N, 3564, 570105								
*9515						6		8
*9528, 9544								8
*9576								
*180101-180103, 180105-1806, 1808, 1812, 1817N, 1823N-1825, 1827, 1828, 1831, 1832, 1834, 1838, 1839, 1842-1845						6		
*180104, 1840						6		
*180701, 180702, 1829, 1833, 1837, 3943						6		
*1809					5	6		
*1810, 1811, 1835								
*1813						6		
*1814						6		
*1815						6		8
*1818						6	7	
*1819, 1830						6		8
*1820						6		
*1821								8
*1822		2				6		
*1826, 1841								
*1836						6		
*2752			3					
*350201-350203, 3583, 3595								
*350301, 350302, 3506, 3513, 3536, 3555, 3556, 3559, 3570, 3574, 3575, 3584, 3585, 3598, 3932, 4071, 4410, 5602, 5604, 5611, 5622				4				
*3505, 3551								
*352001, 352002, 3528								
*3521		2				6		8
*3524, 5714		2				6		
*3538, 3933, 4502, 8201, 8202, <i>Cw*0434</i>				4				
*3558								8
*3566								
Well No.	1	2	3	4	5	6	7	8

Lot No.: **56G**

Lot-specific Information

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75	150	85	105	90	95	105	160	Length of spec. PCR product(s)
					130	205		
9	10	11	12	13	14	15	16	Well No.
								*1558, 1573, 9537
		11						*1575, 9505, 9511N, 9545
9	w	11						*1584, 350801-350804, 3545, 3561, 3562, 3580, 5719
		w					w	*1588
9	10						16	*9507, 3553N, 3564, 570105
		11					16	*9515
		w						*9528, 9544
		11					w	*9576
	10	11				15	16	*180101-180103, 180105-1806, 1808, 1812, 1817N, 1823N-1825, 1827, 1828, 1831, 1832, 1834, 1838, 1839, 1842-1845
	10					15	16	*180104, 1840
	10	11					16	*180701, 180702, 1829, 1833, 1837, 3943
	10	11				15	16	*1809
	10	11				15	16	*1810, 1811, 1835
	w	11				15		*1813
	10	11			14	15	16	*1814
		11				15	16	*1815
	10	11				15	16	*1818
		11				15		*1819, 1830
	10	w				15	16	*1820
						15		*1821
	10	11				15	16	*1822
						15	16	*1826, 1841
9	10	11				15	16	*1836
							16	*2752
9						15	16	*350201-350203, 3583, 3595
9							16	*350301, 350302, 3506, 3513, 3536, 3555, 3556, 3559, 3570, 3574, 3575, 3584, 3585, 3598, 3932, 4071, 4410, 5602, 5604, 5611, 5622
9	10	11			14		16	*3505, 3551
9	10	11				15	16	*352001, 352002, 3528
		11					16	*3521
9	10	11					16	*3524, 5714
9								*3538, 3933, 4502, 8201, 8202, Cw*0434
		11			14		16	*3558
	w	11			14			*3566
9	10	11	12	13	14	15	16	Well No.

Lot No.: **56G**

Lot-specific Information

www.olerup.com

Length of spec.	145	270	135	190	145	205	130	170
PCR product(s)			290			235		
Well No.	1	2	3	4	5	6	7	8
*3572								
*3581		2						
*370101-370105, 370107, 3703N, 3705, 3708, 3715-3718			3				7	
*370106, 3706							7	
*3702			3					
*3704		2	3				7	
*3707			3					8
*3709, 3712, 3719			3					
*3710					5		7	
*3711, 3714							7	
*3713			3				7	8
*3806, 3807, 5110, 5131, 5181	1							
*3819, 3903, 3924, 3937, 4807								
*3909							7	
*4003								
*4020, 4038, 4052, 4059, 4060, 4606								
*4213			3					
*440208								
*4406, 5105, 5108, 5120, 5144N	1							
*4415				4		6		
*4418, 4501, 4504-4507, 5613				4				
*4483					5			
*4503								
*4814								
*490101, 4902, 4906, 500101-5002, 5004-5008, 5625				4				8
*490102				4				8
*4903	1			4				8
*5009, 9533				4				8
*510101-5103, 5106, 5111N, 5112, 5114, 5116-5118, 5121-512402, 5126-5128, 5130, 5132-5135, 5138, 5139, 5141N, 5143, 5148, 5149, 5151, 5152, 5157-5160, 5164-5171, 5174-5177, 5179, 5180, 5183, 5184, 520601, 520602	1							8
*5104, 5146, 5156	1	2						8
*5107, 520101-520105, 5204, 5205, 5207, 5208, 5211-5213, 5215	1							8
Well No.	1	2	3	4	5	6	7	8

Lot No.: **56G**

Lot-specific Information

www.olerup.com

75	150	85	105	90	95	105	160	Length of spec. PCR product(s)
					130	205		
9	10	11	12	13	14	15	16	Well No.
9	10	11			14	15	16	*3572
9						15	16	*3581
						15		*370101-370105, 370107, 3703N, 3705, 3708, 3715-3718
								*370106, 3706
						15	16	*3702
						15		*3704
						15		*3707
						15		*3709, 3712, 3719
						15		*3710
						15		*3711, 3714
						15		*3713
							16	*3806, 3807, 5110, 5131, 5181
					14		16	*3819, 3903, 3924, 3937, 4807
							16	*3909
	10				14		16	*4003
	10						16	*4020, 4038, 4052, 4059, 4060, 4606
								*4213
w								*440208
9								*4406, 5105, 5108, 5120, 5144N
9			12					*4415
9			12					*4418, 4501, 4504-4507, 5613
9						15		*4483
9			12					*4503
	10	11			14		16	*4814
			12				16	*490101, 4902, 4906, 500101-5002, 5004- 5008, 5625
			12				w	*490102
			12			15	16	*4903
							16	*5009, 9533
							16	*510101-5103, 5106, 5111N, 5112, 5114, 5116-5118, 5121-512402, 5126-5128, 5130, 5132-5135, 5138, 5139, 5141N, 5143, 5148, 5149, 5151, 5152, 5157-5160, 5164-5171, 5174-5177, 5179, 5180, 5183, 5184, 520601, 520602
							16	*5104, 5146, 5156
						15	16	*5107, 520101-520105, 5204, 5205, 5207, 5208, 5211-5213, 5215
9	10	11	12	13	14	15	16	Well No.

Length of spec.	145	270	135	190	145	205	130	170
PCR product(s)			290			235		
Well No.	1	2	3	4	5	6	7	8
*510901, 510902, 5119, 5140, 5142, 5173	1							
*511301, 511302, 5162	1			4				8
*5115	1			4				
*5129, 5136, 5155, 5161, 5182	1							8
*5137, 5145, 5163	1					6		8
*5153	1							
*5154					5			
*5172	1							8
*5178					5			8
*5209	1		3					8
*5214	1			4				8
*580101, 580102, 5802, 5804, 5807, 5810N-5816, 5818, 5821, 5823, 5824	1							
*580103	1							
*5805	1							
*5806, 5819	1							8
*5808	1							8
*5809	1	2				6		
*5817N								
*5820	1							
*5822	1							
*7805, 7806								8
A*9288								
Cw*0312, Cw*0319, Cw*0326				w				
Cw*0408, Cw*0527, Cw*1515				4				
Cw*0620, Cw*0781								
Well No.	1	2	3	4	5	6	7	8

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

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA- B*53 subtyping. In addition, wells number 3 to 7, 11 and 15 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 1st, 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

³The nucleotide position, in the 1st, 2nd or 3rd 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.

Lot No.: **56G**

Lot-specific Information

www.olerup.com

75	150	85	105	90	95	105	160	Length of spec. PCR product(s)
					130	205		
9	10	11	12	13	14	15	16	Well No.
9							16	*510901, 510902, 5119, 5140, 5142, 5173
							16	*511301, 511302, 5162
9			12				16	*5115
								*5129, 5136, 5155, 5161, 5182
		11					16	*5137, 5145, 5163
							w	*5153
9								*5154
							w	*5172
							16	*5178
						15	16	*5209
						15	16	*5214
9	10	11		13			16	*580101, 580102, 5802, 5804, 5807, 5810N- 5816, 5818, 5821, 5823, 5824
9	10			13			16	*580103
		11		13			16	*5805
		11		13			16	*5806, 5819
				13			16	*5808
9	10	11		13			16	*5809
9	10	11		13			16	*5817N
				13			16	*5820
9	w	11		13				*5822
						15	16	*7805, 7806
	w							A*9288
								Cw*0312, Cw*0319, Cw*0326
								Cw*0408, Cw*0527, Cw*1515
		w						Cw*0620, Cw*0781
9	10	11	12	13	14	15	16	Well No.

⁴Primer mix 3: Specific PCR fragment of 135 bp in the B*5303, 5305 and 5316 and the B*2752, 370101-370105, 370107-3705, 3707-3709, 3712, 3713, 3715-3719 and 5209 alleles. Specific PCR fragment of 290 bp in the B*5315 and the B*4213 alleles.

Primer mix 6: Specific PCR fragment of 205 bp in the B*5302 and 5306 and the B*153801, 153802, 180101-1809, 1812-1815, 1817N-1820, 1822-1825, 1827-1834, 1836-1840, 1842-1845, 3521, 3524, 3943, 5137, 5145, 5163, 5714 and 5809 alleles. Specific PCR fragment of 235 bp in the the B*5305 and 5312 and the B*1325, 9515 and 4415 alleles.

Primer mix 14: Specific PCR fragment of 95 bp in the B*5314 and the B*0709, 0711, 0717, 0828, 0835, 0837, 1405, 1507, 1555, 1568, 1814, 3505, 3551, 3558, 3566, 3572, 3819, 3903, 3924, 3937, 4003, 4206, 4209, 4612, 4807 and 4814 and the Cw*0808 alleles. Specific PCR fragment of 130 bp in the B*5310 allele.

Primer mix 15: Specific PCR fragment of 105 bp in the B*5309 and 5317 and the B*180101-1806, 1808-1815, 1817N-1828, 1830-1832, 1834-1836, 1838-1845, 352001, 352002, 3528, 3572, 3595, 370101-370105, 370107-3705, 3707-3719, 4483, 4903, 5107, 520101-520105, 5203-5205, 5207-5215, 7805 and 7806 alleles. Specific PCR fragment of 205 bp in the B*5319 and the B*350201-350203, 3581, 3583 and 3595 alleles.

⁵The B*5319, B*5203 and B*5210 give rise to identical amplification patterns with the HLA-B*53 subtyping kit. These three alleles can be distinguished by the HLA-B low resolution kit and/or the HLA-B*52 subtyping kit.

'w', might be weakly amplified.

CELL LINE VALIDATION SHEET																			
HLA-B*53 SSP subtyping kit																			
			Prod. No.:	Well															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	IHC cell line	B*		200966401	200966402	200966403	200966404	200966405	200966406	200966407	200966408	200966409	200966410	200966411	200966412	200966413	200966414	200966415	200966416
1	9001 SA	*0702		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
2	9280 LK707	*5201	*7301	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+
3	9011 E4181324	*52011		+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+
4	9275 GU373	*1510	*5301	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+
5	9009 KAS011	*3701		-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-
6	9353 SM	*3901	*5101	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
7	9020 QBL	*1801		-	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+
8	9025 DEU	*3501		-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+
9	9026 YAR	*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
10	9107 LKT3	*5401		-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
11	9051 PITOUT	*4403		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
12	9052 DBB	*5701		-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+
13	9004 JESTHOM	*2705		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
14	9071 OLGA	*1501	*1520	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+
15	9075 DKB	*4001		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
16	9037 SWEIG007	*4002		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
17	9282 CTM3953540	*0801	*5501	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+
18	9257 32367	*1401	*5601	-	-	-	+	-	-	-	+	+	-	-	+	-	-	-	+
19	9038 BM16	*1801		-	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+
20	9059 SLE005	*4001		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
21	9064 AMALA	*1501		-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
22	9056 KOSE	*3503		-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+
23	9124 IHL	*4002	*5602	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+
24	9035 JBUSH	*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
25	9049 IBW9	*1402		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
26	9285 WT49	*5801		+	-	-	-	-	-	-	-	+	+	+	-	+	-	-	+
27	9191 CH1007	*0705	*5101	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
28	9320 BEL5GB	*4402	*4403	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
29	9050 MOU	*4403		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
30	9021 RSH	*4201		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
31	9019 DUCAF	*1801		-	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+
32	9297 HAG	*4102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
33	9098 MT14B	*4001		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
34	9104 DHIF	*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
35	9302 SSTO	*4402		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
36	9024 KT17	*1501	*3501	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+
37	9065 HHKB	*0702		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
38	9099 LZL	*1501		-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
39	9315 CML	*0801	*2705	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
40	9134 WHONP199	*1302	*4601	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	+
41	9055 H0301	*1402		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
42	9066 TAB089	*4601		-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
43	9076 T7526	*4601		-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
44	9057 TEM	*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
45	9239 SHJO	*4201	*5001	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
46	9013 SCHU	*0702		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
47	9045 TUBO	*5101		+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
48	9303 TER-ND	*3501	*4403	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+

CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-B*53 SSP

Product number: 101.549-06 – including *Taq* polymerase
Lot number: 56G
Expiry date: 2011-December-01
Number of tests: 6
Number of wells per test: 16

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2009-664-01	9	2009-664-09
2	2009-664-02	10	2009-664-10
3	2009-664-03	11	2009-664-11
4	2009-664-04	12	2009-664-12
5	2009-664-05	13	2009-664-13
6	2009-664-06	14	2009-664-14
7	2009-664-07	15	2009-664-15
8	2009-664-08	16	2009-664-16

The specificity of each primer solution of the HLA-B*53 primer set has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 2 and 5 were available. The specificities of the primers in primer solutions 2 and 5 were tested by separately adding one additional 3'-primer, respectively one additional 5'-primer. In primer solutions 4, 6 and 14 one of the 3'-primers was not possible to test. Additional primers in primer solutions 3, 6 and 14 were tested by separately adding one additional 3'-primer or one additional 5'-primer.

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

Date of approval: 2009-December-21

Approved by:

Quality Control, Supervisor

Lot No.: **56G**

Lot-specific Information

www.olerup.com

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*53
Product number: 101.549-06
Lot number: 56G

Intended use: HLA-B*53 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 III, 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.

Saltsjöbaden, Sweden
2009-December-21

Olle Olerup
Managing Director

Lot No.: **56G**

Lot-specific Information

www.olerup.com

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