

***Olerup SSP*[®] HLA-B*54**

Product number:	101.569-06 – including <i>Taq</i> polymerase
Lot number:	08H
Expiry date:	2012-April-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. 08H.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] HLA-B*54 LOT

The HLA-B*54 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP*[®] HLA-B*54 lot was made (**Lot No. 41F**).

The primers of the wells detailed below have been added, exchanged or modified.

Well	5'-primer	3'-primer	rationale
4	Added	-	Increased yield of specific PCR product, primer added for the B*5419 allele.
11	Added	Added	Primer pair added for the B*5418 allele.

PRODUCT DESCRIPTION

HLA-B*54 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*5401 to B*5419 alleles.

PLATE LAYOUT

Each test consists of 16 PCR reactions in a 16 well 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*54' in silver/gray ink.

Well No. 1 is marked with the Lot Number '08H'.

A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.

The PCR plates are covered with a PCR-compatible foil.

Please note: When removing each 16 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

The interpretation of HLA-B*54 SSP subtypings will be influenced by eight B*07, seven B*08, most B*13, most B*15, the B*18, eight B*27, most B*35, the B*370104, most B*38, most B*39, most B*40, six B*41, two B*42, twelve B*44, ten B*45, the B*46, most B*48, the B*49, the B*50, most B*51, most B*52, most B*53, most B*55, most B*56, many B*57, the B*58, most B*59, the B*67, the B*78, the B*81 and B*82 alleles when present on the other haplotype. In addition, the Cw*0203 allele will be amplified by primer mix 9 and the Cw*0530 allele will be amplified by primer mix 3.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*54 alleles, i.e. **B*5401 to B*5419**, recognized by the HLA Nomenclature Committee in January 2010¹ will give rise to unique amplification patterns by the primers in the HLA-B*54 subtyping kit.

¹HLA-B alleles listed on the IMGT/HLA web page 2010-January-16, release 2.28.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 19 HLA-B*54 alleles can be combined in 190 homozygous and heterozygous combinations. Eighty-three of these genotypes do not give rise to unique amplification patterns. The different sizes of the specific PCR products generated by primer mixes 6 and 11 were not considered in these calculations.

+++++--	-----	5402, 5408N = 5408N, 5419
+++++---	-----	5402, 5403 = 5403, 5419
++++-+-	--+-----	5402, 5411 = 5411, 5419
++++-+-	-----+	5402, 5415 = 5415, 5419
++++-+-	-----	5402, 5404 = 5404, 5419
++++---+	+-----	5402, 5409 = 5409, 5419
++++---+	-----+	5402, 5414 = 5414, 5419
++++---+	-----	5402, 5406 = 5406, 5419
++++---	-+-----	5402, 5410 = 5410, 5419
++++---	--+-----	5402, 5418 = 5418, 5419
++++---	---+-----	5402, 5412 = 5412, 5419
++++---	-----+	5402, 5413 = 5413, 5419
++++---	-----+	5402, 5407 = 5407, 5419
++++---	-----+	5402, 5416 = 5416, 5419
++++---	-----+	5402, 5417 = 5417, 5419
++++---	-----	5401, 5402 = 5401, 5419 = 5402, 5419 = 5419, 5419
+++-+-	--+-----	5403, 5411 = 5408N, 5411 = 5408N, 5418
+++-+-	-----+	5403, 5415 = 5408N, 5415 = 5408N, 5416
+++-+-	-----	5401, 5408N = 5403, 5404 = 5403, 5408N = 5404, 5408N = 5408N, 5408N
+++-+-	-----	5401, 5403 = 5403, 5403
+++-+-	--+-----	5411, 5416 = 5415, 5418
+++-+-	--+-----	5401, 5411 = 5404, 5418 = 5411, 5418
+++-+-	-----+	5401, 5415 = 5404, 5416 = 5415, 5416
+++---+	-----+	5401, 5414 = 5406, 5416 = 5414, 5416
+++---	-+-----	5401, 5410 = 5410, 5410
+++---	--+-----	5401, 5418 = 5418, 5418
+++---	---+-----	5401, 5412 = 5412, 5412
+++---	-----+	5401, 5413 = 5413, 5413
+++---	-----+	5401, 5407 = 5407, 5407
+++---	-----+	5401, 5416 = 5416, 5416
+++---	-----+	5401, 5417 = 5417, 5417
+--+-+	-----+	5404, 5414 = 5406, 5415 = 5414, 5415
+--+-	--+-----	5404, 5411 = 5411, 5411
+--+-	-----+	5404, 5415 = 5415, 5415
+--+-+	+-----	5406, 5409 = 5409, 5409
+--+-+	-----+	5406, 5414 = 5414, 5414

SPECIFICITY TABLE

HLA-B*54 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-B*54 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*54 alleles	Other amplified HLA Class I alleles ³
1	215 bp	800 bp	*5401-5419	*070219, 150104, 370104, 4094
2⁴	105 bp	1070 bp	*5401-5403, 5405N, 5407, 5408N, 5410, 5412, 5413, 5416-5419	*0778, 130201-1303, 1308Q, 1309, 1314-1316, 1318, 1319, 1327, 1330-1334, 1542, 3560, 4415, 4418, 4501, 4503-4508, 4510, 4511, 4611, 4618, 490101-4903, 4906-4909, 500101-5002, 5004-5008, 5115, 550101-550103, 550105-5503, 5505, 5507, 5509-5512, 5515, 5516, 5518, 5519, 5521, 5522, 5524-5526, 5529-5531, 5533-5538, 560101-560103, 5607, 5608, 5613, 5614, 5616, 5617, 5619N, 5620, 5623-5627, 5901, 5904, 5905
3⁵	160 bp	1070 bp	*5401, 5403, 5404, 5406-5419	*4424, 5139, Cw*0530
4⁶	160 bp	1070 bp	*5402, 5419	*0713, 3811, 6702
5	180 bp	1070 bp	*5403, 5408N	*1303, 1573, 4071, 4410, 4415, 4418, 4501, 4504-4507, 4511, 4611, 490101-4903, 4906, 4908, 4909, 500101-5002, 5004-5008, 5115, 5162, 560101-5602, 5604, 5607, 5608, 5613, 5614, 5616, 5617, 5620, 5624-5627, 5904, 8201-8203
6^{4,7}	105 bp, 235 bp	1070 bp	*5404, 5408N, 5411, 5415	*0784, 0809, 1335, 1583, 2714, 40060101-400602, 4044, 4053, 4070, 4075, 4083, 4093, 4095, 4096, 4101, 4105-4107, 4109, 4112, 4204, 4420, 4447, 510101-5103, 5105, 5107-5112, 5114, 5116-512403, 5126-5134, 5136, 5138-5141N, 5143,

Lot No.: **08H**

Lot-specific information

www.olerup-ssp.com

				5144N, 5148-5155, 5157, 5158, 5160, 5161, 5165-5180, 5182-5189, 520101-5213, 5215-5219, 5517, 5520, 5527, 5528, 560501-5606, 5615, 5621, 5808, 5902, 7801-7806
7⁴	140 bp	1070 bp	*5405N	
8	280 bp	1070 bp	*5406, 5409, 5414	*0709, 0711, 0717, 0828, 0835, 0837, 1304, 1310, 1326, 15010101-150104, 150106-1508, 151101-1516, 151801-1521, 1523-1529, 1531-1536, 153801-1540, 1543, 1544, 1546, 1547, 1549-1557, 1560-1562, 1564-1572, 1574-1576, 157801-1582, 1584, 1585, 1587-1589, 1591-1598, 9501-9529, 9531, 9532, 9534-9536, 9538-9549N, 9551-9561, 9563-9567, 9569-9576, 9578, 9581N-9586, 180101-1815, 1817N-1825, 1827-1840, 1842-1847, 2741, 350101-350119, 3505, 3507-350804, 3510, 3511, 351401-3517, 3519-3521, 3523-3530, 3532, 3535, 3537, 3540N-3543, 3545-3554, 3557, 3558, 3561-3564, 3566-3569, 3571, 3572, 3576-3580, 3582, 3586, 3589-3594, 3597, 3599, 3907, 3943, 4003, 4020, 4038, 4052, 4059, 4060, 4209, 4417, 4443, 4509, 460101-4610, 4612-4617, 4619-4622, 480201, 480202, 4814, 4904, 4905, 5137, 5145, 5163, 530101-5303, 5305, 5306, 530801-5318, 5320, 5321, 5514, 5523, 5532, 5603, 5609, 5618, 570101-570106, 5706, 5708, 5710, 5711, 5713-5716, 5718-5727, 5729, 580101-5802, 5804-5807, 5809-5819, 5821-5826
9	200 bp	1070 bp	*5409	*0748, 0813, 0825, 0840 ^w , 1310, 1311, 150501, 150502, 1531, 1548, 1552, 1555, 1591, 9507, 9514, 9523, 9524, 9536,

Lot No.: **08H**

Lot-specific information

www.olerup-ssp.com

				9551, 9585, 9586, 180101-180105, 180107, 180108, 1803-180702, 1809-1812, 1813 ^w , 1814, 1817N, 1820, 1823N-1826, 1828, 1829, 1831-1847, 2707, 2711, 2732-2734, 2743, 3505, 3522, 3531, 3532, 3551, 3566 ^w , 356802, 3572, 3589, 3597, 380101-3809, 3811-3819, 3821, 39010101, 39010102L, 390103-390502, 3907, 3910, 3912-3917, 391901-3920, 3922, 3923, 3925N-3932, 3937-3941, 3943-3949, 3951-3956, 400101-400103, 400105-400208, 4003, 4007-401403, 4018-4022N, 4024, 4025, 4027, 4029, 4031, 4033, 4035-4040, 4042, 4043, 4045-4050, 4052, 4054-4058, 4061-4063, 4065-4067, 4069, 4071-4074, 4076-4082, 4084, 4085, 4087-4092, 4094, 4097, 4099, 4431, 4454, 4465, 4482, 4606, 461301, 461302, 4619, 4621, 480101, 480102, 480301, 4804, 4806, 4807, 4809-4814, 4816, 4818, 4820, 5181, 5314, 5504, 5508, 5602, 5604, 5610, 5618, 5816, 5818, 5820, 5827, 670101-6702, 8101-8104N, Cw*0203
10	225 bp	1070 bp	*5410	*0778, 0784, 1318, 1331, 1504, 9537, 3933, 5105, 5129, 5154, 5161, 5182, 5509, 5521, 5537
11⁸	185 bp, 210 bp	1070 bp	*5411, 5418	
12	150 bp	1070 bp	*5412	
13⁴	135 bp	1070 bp	*5413	
14	210 bp	800 bp	*5407	
15⁴	95 bp	1070 bp	*5414-5416	*0784, 1304, 1335, 1504, 1516, 1567, 1595, 9555, 3537, 4044, 4420, 4447, 4509, 4904, 4905, 5137, 5212, 5523, 5527
16	340 bp	1070 bp	*5417	

Lot No.: 08H

Lot-specific information

www.olerup-ssp.com

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*54 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 length of the specific PCR product(s) of the alleles amplified by these primer mixes is 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*54 subtyping.

In addition, well 14 contains the primer pair giving rise to the shorter, 800 bp, internal positive control 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 non-HLA-B*54 alleles will be amplified by primer mixes 1 to 6, 8 to 10 and 15. In addition, the Cw*0203 allele will be amplified by primer mix 9 and the Cw*0530 allele will be amplified by primer mix 3.

⁴ Specific PCR fragments shorter than 150 base pairs have a lower intensity than longer PCR bands.

⁵ Primer mix 3 may give a lower yield of specific PCR product than the other B*54 primer mixes.

⁶ Primer mix 4 may have tendencies of non-specific amplification.

⁷ Primer mix 6: Specific PCR fragment of 105 bp in the B*5404, 5411 and 5415 and the B* 0784, 0809, 1335, 1583, 2714, 40060101-400602, 4044, 4053, 4070, 4075, 4083, 4093, 4095, 4096, 4101, 4105-4107, 4109, 4112, 4204, 4420, 4447, 510101-5103, 5105, 5107-5112, 5114, 5116-512403, 5126-5134, 5136, 5138-5141N, 5143, 5144N, 5148-5155, 5157, 5158, 5160, 5161, 5165-5180, 5182-5189, 520101-5213, 5215-5219, 5517, 5520, 5527, 5528, 560501-5606, 5615, 5621, 5808, 5902 and B*7801-7806 alleles. Specific PCR fragment of 235 bp in the B*5408N allele.

⁸ Primer mix 11: Specific PCR fragment of 185 bp in the B* 5411 allele. Specific PCR fragment of 210 bp in the B* 5418 allele.

‘w’, may be weakly amplified.

INTERPRETATION TABLE

HLA-B*54 SSP subtyping

Amplification patterns of the B*5401 to 5419 alleles

	Well ⁴							
	1	2	3	4	5	6	7	8
Length of spec.	215	105	160	160	180	105	140	280
PCR product						235		
Length of int.	800	1070	1070	1070	1070	1070	1070	1070
pos. control ¹								
5'-primer ²	1 st I	357	106	103	420	357	106	419
	5' -CAg 3'	5' -Tgg 3'	5' -CCA 3'	5' -CCT 3'	5' -TTA 3'	5' -Tgg 3'	5' -CCA 3'	5' -gTC 3'
				103				
				5' -CCT 3'				
				110				
				5' -gTT 3'				
3'-primer(s) ³	175	420	226	226	553	419	207	3 rd I
	5' -CCg 3'	5' -gCT 3'	5' -CAC 3'	5' -CAC 3'	5' -CTA 3'	5' -CgT 3'	5' -TCC 3'	5' -TAT 3'
					559	553		
					5' -CAg 3'	5' -CTA 3'		
Well No.	1	2	3	4	5	6	7	8
HLA-B allele								
*5401	1	2	3					
*5402	1	2		4				
*5403	1	2	3		5			
*5404	1		3			6		
*5405N	1	2					7	
*5406	1		3					8
*5407	1	2	3					
*5408N	1	2	3		5	6		
*5409	1		3					8
*5410	1	2	3					
*5411	1		3			6		
*5412	1	2	3					
*5413	1	2	3					
*5414	1		3					8
*5415	1		3			6		
*5416	1	2	3					
*5417	1	2	3					
*5418	1	2	3					
*5419	1	2	3	4				
*070219, 370104	1							
Well No.	1	2	3	4	5	6	7	8

INTERPRETATION TABLE								
HLA-B*54 SSP subtyping								
Amplification patterns of the B*5401 to 5419 alleles								
Well ⁴								
9	10	11	12	13	14	15	16	
200	225	185	150	135	210	95	340	Length of spec.
		210						PCR product
1070	1070	1070	1070	1070	800	1070	1070	Length of int.
								pos. control ¹
369	357	89	206	133	106	357	709	5'-primer ²
5' -TAC 3'	5' -Tgg 3'	5' -gAA 3'	5' -A gg 3'	5' -CCg 3'	5' -CCA 3'	5' -Tgg 3'	5' -A gg 3'	
		357						
		5' -Tgg 3'						
527	539	259	317	226	275	412	916	3'-primer(s) ³
5' -CCA 3'	5' -TCC 3'	5' -gTT 3'	5' -ggA 3'	5' -CAC 3'	5' -CCA 3'	5' -gTC 3'	5' -gAT 3'	
527		499				412		
5' -CCA 3'		5' -ggA 3'				5' -gTC 3'		
9	10	11	12	13	14	15	16	Well No.
								HLA-B allele
								*5401
								*5402
								*5403
								*5404
								*5405N
								*5406
					14			*5407
								*5408N
9								*5409
	10							*5410
		11						*5411
			12					*5412
				13				*5413
						15		*5414
						15		*5415
						15		*5416
							16	*5417
		11						*5418
								*5419
								*070219, 370104
9	10	11	12	13	14	15	16	Well No.

Lot No.: **08H**

Lot-specific information

www.olerup-ssp.com

Length of spec.	215	105	160	160	180	105	140	280
PCR product						235		
Well No.	1	2	3	4	5	6	7	8
*0709, 0711, 0717, 0828, 0835, 0837, 1326, 15010101-150103, 150106-150303, 1506-1508, 151101-1515, 151801-1521, 1523-1529, 1532-1536, 153801-1540, 1543, 1544, 1546, 1547, 1549-1551, 1553, 1554, 1556, 1557, 1560-1562, 1564-1566, 1568-1572, 1574-1576, 157801-1582, 1584, 1585, 1587-1589, 1592-1594N, 1596-1598, 9501-9506, 9508-9513, 9515-9522, 9525-9529, 9531, 9532, 9534, 9535, 9538-9549N, 9552-9554, 9556-9561, 9563-9567, 9569-9576, 9578, 9581N-9584, 180106, 1802, 1808, 1815, 1818, 1819, 1821, 1822, 1827, 1830, 2741, 350101-350119, 3507-350804, 3510, 3511, 351401-3517, 3519-3521, 3523-3530, 3535, 3540N-3543, 3545-3550, 3552-3554, 3557, 3558, 3561-3564, 3567, 356801, 3569, 3571, 3576-3580, 3582, 3586, 3590-3594, 3599, 4059, 4060, 4209, 4417, 4443, 460101-4605, 4607N-4610, 4612, 4614-4617, 4620, 4622, 480201, 480202, 5145, 5163, 530101-5303, 5305, 5306, 530801-5313, 5315-5318, 5320, 5321, 5514, 5532, 5603, 5609, 570101-570106, 5706, 5708, 5710, 5711, 5713-5716, 5718-5727, 5729, 580101-5802, 5804-5807, 5809-5815, 5817N, 5819, 5821-5826								8
*0713				4				
*0748, 0813, 0825, 1311, 1548, 1826, 1841, 2707, 2711, 2732-2734, 2743, 3522, 3531, 380101-3809, 3812-3819, 3821, 39010101, 39010102L, 390103-390502, 3910, 3912-3917, 391901-3920, 3922, 3923, 3925N-3932, 3937-3941, 3944-3949, 3951-3956, 400101-400103, 400105-400208, 4007-401403, 4018, 4019, 4021, 4022N, 4024, 4025, 4027, 4029, 4031, 4033, 4035-4037, 4039, 4040, 4042, 4043, 4045-4050, 4054-4058, 4061-4063, 4065-4067, 4069, 407201-4074, 4076-4082, 4084, 4085, 4087-4092, 4097, 4099, 4431, 4454, 4465, 4482, 480101, 480102, 480301, 4804, 4806, 4807, 4809-4813, 4816, 4818, 4820, 5181, 5504, 5508, 5610, 5820, 5827, 670101, 670102, 8101-8104N, Cw*0203								
*0778, 1318, 1331, 5509, 5521, 5537		2						
*0784						6		
Well No.	1	2	3	4	5	6	7	8

Lot No.: **08H**

Lot-specific information

www.olerup-ssp.com

200	225	185	150	135	210	95	340	Length of spec.
		210						PCR product
9	10	11	12	13	14	15	16	Well No.
								*0709, 0711, 0717, 0828, 0835, 0837, 1326, 15010101-150103, 150106-150303, 1506-1508, 151101-1515, 151801-1521, 1523-1529, 1532-1536, 153801-1540, 1543, 1544, 1546, 1547, 1549-1551, 1553, 1554, 1556, 1557, 1560-1562, 1564-1566, 1568-1572, 1574-1576, 157801-1582, 1584, 1585, 1587-1589, 1592-1594N, 1596-1598, 9501-9506, 9508-9513, 9515-9522, 9525-9529, 9531, 9532, 9534, 9535, 9538-9549N, 9552-9554, 9556-9561, 9563-9567, 9569-9576, 9578, 9581N-9584, 180106, 1802, 1808, 1815, 1818, 1819, 1821, 1822, 1827, 1830, 2741, 350101-350119, 3507-350804, 3510, 3511, 351401-3517, 3519-3521, 3523-3530, 3535, 3540N-3543, 3545-3550, 3552-3554, 3557, 3558, 3561-3564, 3567, 356801, 3569, 3571, 3576-3580, 3582, 3586, 3590-3594, 3599, 4059, 4060, 4209, 4417, 4443, 460101-4605, 4607N-4610, 4612, 4614-4617, 4620, 4622, 480201, 480202, 5145, 5163, 530101-5303, 5305, 5306, 530801-5313, 5315-5318, 5320, 5321, 5514, 5532, 5603, 5609, 570101-570106, 5706, 5708, 5710, 5711, 5713-5716, 5718-5727, 5729, 580101-5802, 5804-5807, 5809-5815, 5817N, 5819, 5821-5826
								*0713
9								*0748, 0813, 0825, 1311, 1548, 1826, 1841, 2707, 2711, 2732-2734, 2743, 3522, 3531, 380101-3809, 3812-3819, 3821, 39010101, 39010102L, 390103-390502, 3910, 3912-3917, 391901-3920, 3922, 3923, 3925N-3932, 3937-3941, 3944-3949, 3951-3956, 400101-400103, 400105-400208, 4007-401403, 4018, 4019, 4021, 4022N, 4024, 4025, 4027, 4029, 4031, 4033, 4035-4037, 4039, 4040, 4042, 4043, 4045-4050, 4054-4058, 4061-4063, 4065-4067, 4069, 407201-4074, 4076-4082, 4084, 4085, 4087-4092, 4097, 4099, 4431, 4454, 4465, 4482, 480101, 480102, 480301, 4804, 4806, 4807, 4809-4813, 4816, 4818, 4820, 5181, 5504, 5508, 5610, 5820, 5827, 670101, 670102, 8101-8104N, Cw*0203
	10							*0778, 1318, 1331, 5509, 5521, 5537
	10					15		*0784
9	10	11	12	13	14	15	16	Well No.

Lot No.: **08H**

Lot-specific information

www.olerup-ssp.com

Length of spec.	215	105	160	160	180	105	140	280
PCR product						235		
Well No.	1	2	3	4	5	6	7	8
*0809, 1583, 2714, 40060101-400602, 4053, 4070, 4075, 4083, 4093, 4095, 4096, 4101, 4105-4107, 4109, 4112, 4204, 510101-5103, 5107-5112, 5114, 5116-512403, 5126-5128, 5130-5134, 5136, 5138, 5140, 5141N, 5143, 5144N, 5148-5153, 5155, 5157, 5158, 5160, 5165-5180, 5183-5189, 520101-5211, 5213, 5215-5219, 5517, 5520, 5528, 560501-5606, 5615, 5621, 5808, 5902, 7801-7806						6		
*0840								
*130201-130206, 1308Q, 1309, 1314-1316, 1319, 1327, 1330, 1332-1334, 1542, 3560, 4503, 4508, 4510, 4618, 4907, 550101-550103, 550105-5503, 5505, 5507, 5510-5512, 5515, 5516, 5518, 5519, 5522, 5524-5526, 5529-5531, 5533-5536, 5538, 5619N, 5623, 5901, 5905		2						
*1303, 4415, 4418, 4501, 4504-4507, 4511, 4611, 490101-4903, 4906, 4908, 4909, 500101-5002, 5004-5008, 5115, 560101-560103, 5607, 5608, 5613, 5614, 5616, 5617, 5620, 5624-5627, 5904		2			5			
*1304, 1516, 1567, 1595, 9555, 3537, 4509, 4904, 4905, 5137, 5523								8
*1310, 150501, 150502, 1531, 1552, 1555, 1591, 9507, 9514, 9523, 9524, 9536, 9551, 9585, 9586, 180101-180105, 180107, 180108, 1803-180702, 1809-1812, 1814, 1817N, 1820, 1823N-1825, 1828, 1829, 1831-1840, 1842-1847, 3505, 3532, 3551, 356802, 3572, 3589, 3597, 3907, 3943, 4003, 4020, 4038, 4052, 4606, 461301, 461302, 4619, 4621, 4814, 5314, 5618, 5816, 5818								8
*1335, 4044, 4420, 4447, 5212, 5527						6		
*150104	1							8
*1504								8
*1573, 4410, 5162, 8201-8203					5			
*9537, 3933								
*1813, 3566								8
*3811, 6702				4				
*4071, 5602, 5604					5			
*4094	1							
*4424, Cw*0530			3					
*5105, 5129, 5154, 5161, 5182						6		
*5139			3			6		
HLA-B allele								
Well No.	1	2	3	4	5	6	7	8

200	225	185	150	135	210	95	340	Length of spec.
		210						PCR product
9	10	11	12	13	14	15	16	Well No.
								*0809, 1583, 2714, 40060101-400602, 4053, 4070, 4075, 4083, 4093, 4095, 4096, 4101, 4105-4107, 4109, 4112, 4204, 510101-5103, 5107-5112, 5114, 5116-512403, 5126-5128, 5130-5134, 5136, 5138, 5140, 5141N, 5143, 5144N, 5148-5153, 5155, 5157, 5158, 5160, 5165-5180, 5183-5189, 520101-5211, 5213, 5215-5219, 5517, 5520, 5528, 560501-5606, 5615, 5621, 5808, 5902, 7801-7806
w								*0840
								*130201-130206, 1308Q, 1309, 1314-1316, 1319, 1327, 1330, 1332-1334, 1542, 3560, 4503, 4508, 4510, 4618, 4907, 550101-550103, 550105-5503, 5505, 5507, 5510-5512, 5515, 5516, 5518, 5519, 5522, 5524-5526, 5529-5531, 5533-5536, 5538, 5619N, 5623, 5901, 5905
								*1303, 4415, 4418, 4501, 4504-4507, 4511, 4611, 490101-4903, 4906, 4908, 4909, 500101-5002, 5004-5008, 5115, 560101-560103, 5607, 5608, 5613, 5614, 5616, 5617, 5620, 5624-5627, 5904
						15		*1304, 1516, 1567, 1595, 9555, 3537, 4509, 4904, 4905, 5137, 5523
9								*1310, 150501, 150502, 1531, 1552, 1555, 1591, 9507, 9514, 9523, 9524, 9536, 9551, 9585, 9586, 180101-180105, 180107, 180108, 1803-180702, 1809-1812, 1814, 1817N, 1820, 1823N-1825, 1828, 1829, 1831-1840, 1842-1847, 3505, 3532, 3551, 356802, 3572, 3589, 3597, 3907, 3943, 4003, 4020, 4038, 4052, 4606, 461301, 461302, 4619, 4621, 4814, 5314, 5618, 5816, 5818
						15		*1335, 4044, 4420, 4447, 5212, 5527
								*150104
	10					15		*1504
								*1573, 4410, 5162, 8201-8203
	10							* 9537, 3933
w								*1813, 3566
9								*3811, 6702
9								*4071, 5602, 5604
9								*4094
								*4424, Cw*0530
	10							*5105, 5129, 5154, 5161, 5182
								*5139
								HLA-B allele
9	10	11	12	13	14	15	16	Well No.

Lot No.: 08H

Lot-specific information

www.olerup-ssp.com

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*54 subtyping.

In addition, well 14 contains the primer pair giving rise to the shorter, 800 bp, internal positive control in order to allow kit identification.

²The codon, in the 2nd or 3rd exon or the 1st intron, 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 codon, in the 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.

⁴Primer mix 6: Specific PCR fragment of 105 bp in the B*5404, 5411 and 5415 and the B* 0784, 0809, 1335, 1583, 2714, 40060101-400602, 4044, 4053, 4070, 4075, 4083, 4093, 4095, 4096, 4101, 4105-4107, 4109, 4112, 4204, 4420, 4447, 510101-5103, 5105, 5107-5112, 5114, 5116-512403, 5126-5134, 5136, 5138-5141N, 5143, 5144N, 5148-5155, 5157, 5158, 5160, 5161, 5165-5180, 5182-5189, 520101-5213, 5215-5219, 5517, 5520, 5527, 5528, 560501-5606, 5615, 5621, 5808, 5902 and B*7801-7806 alleles. Specific PCR fragment of 235 bp in the B*5408N allele.

Primer mix 11: Specific PCR fragment of 185 bp in the B* 5411 allele. Specific PCR fragment of 210 bp in the B* 5418 allele.

'w', may be weakly amplified.

CELL LINE VALIDATION SHEET																				
HLA-B*54 SSP subtyping kit																				
					Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.:	200508501	200508502	200508503	201071104	200623405	200623406	200737207	200508508	200737209	200737210	201071111	200737212	200737213	200737214	200854415	200854416
	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	*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*54 SSP

Product number: 101.569-06 – including *Taq* polymerase
Lot number: 08H
Expiry date: 2012-April-01
Number of tests: 6
Number of wells per test: 16

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2005-085-01	9	2007-372-09
2	2005-085-02	10	2007-372-10
3	2005-085-03	11	2010-711-11
4	2010-711-04	12	2007-372-12
5	2006-234-05	13	2007-372-13
6	2006-234-06	14	2007-372-14
7	2007-372-07	15	2008-544-15
8	2005-085-08	16	2008-544-16

The specificity of each primer solution of the kit has been tested against 48 well characterized cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 4, 7, 11 to 14 and 16 were available. The specificities of the primers in primer solutions 4, 11 and 12 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 7 and 14 it was only possible to test the 5'-primer, the 3'-primers were not possible to test. In primer solutions 13 and 16 it was only possible to test the 3'-primer, the 5'-primers were not possible to test. In addition, one 3'-primer in primer solution 15 was tested by separately adding an additional 5'-primer. One 5'-primer in primer solution 4 and one 3'-primer in primer solution 5 and 6 was not possible to test.

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

Date of approval: 2010-May-11

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*54
Product number: 101.569-06
Lot number: 08H

Intended use: HLA-B*54 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.

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
2010-May-11

Olle Olerup
Managing Director

Lot No.: **08H**

Lot-specific information

www.olerup-ssp.com

ADDRESSES:

Manufacturer:

Olerup SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

Tel: +46-8-717 88 27

Fax: +46-8-717 88 18

E-mail: info-ssp@olerup.com

Web page: <http://www.olerup-ssp.com>

Distributed by:

Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.

Tel: +43-1-710 15 00

Fax: +43-1-710 15 00 10

E-mail: support-at@olerup.com

Web page: <http://www.olerup.com>

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382

Tel: 1-877-OLERUP1

Fax: 610-344-7989

E-mail: info.us@olerup.com

Web page: <http://www.olerup.com>

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.