

Lot No.: **03F**

Lot-specific information

***Olerup SSP*[®] HLA-B*48**

Product number: 101.546-06u – without *Taq* polymerase
Lot number: 03F
Expiry date: 2010-July-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. 03F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] HLA-B*48 LOT.

The HLA-B*48 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP*[®] HLA-B*48 lot was made (**Lot No. X76**).

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

Well	5'-primer	3'-primer	rationale
5	Modified	-	Modified primer added for the B*4817 allele.
15	Added	Added	Primer pair added for the B*4818 allele.
16	Modified	-	Increased specificity of specific primer pair.

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PRODUCT DESCRIPTION

HLA-B*48 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*4801 to B*4819 alleles.

PLATE LAYOUT

Each HLA-B*48 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*48'.

Well No. 1 is marked with the Lot No. '03F'.

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*48 SSP subtypings will be influenced by the B*07, most B*08, the B*13, the B*14, many B*15, most B*18, several B*27, the B*35, the B*37, the B*38, the B*39, the B*40, the B*41, the B*42, the B*44, the B*45, the B*49, the B*50, the B*51, the B*52, the B*53, the B*54, the B*55, the B*56, the B*57, most B*58, the B*59, the B*67, the B*7301, the B*81, the B*82 and the B*8301 alleles when present on the other haplotype.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*48, i.e. **B*4801 to B*4819**, recognized by the HLA Nomenclature Committee in July 2008¹ will be amplified by the primers in the HLA-B*48 SSP kit.

The B*48 primer set cannot separate the B*4806 and B*8102 alleles. These two alleles can be distinguished by the HLA-B low resolution and/or HLA-B*81 kits.

The B*48 primer set cannot separate the B*4810 and B*0817 alleles. These two alleles can be distinguished by the HLA-B low resolution and/or HLA-B*08 kits.

The B*48 primer set cannot separate the B*4813 and B*3803, 390201-390202, 3908 and 391301-391302 alleles. The B*4813 allele can be distinguished from the B*3803 and B*39xx alleles by the HLA-B low resolution and/or HLA-B*38/HLA-B*39 kits.

The B*48 primer set cannot separate the B*480101 and B*480102 alleler or the B*480301 and B*480302 alleles.

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

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RESOLUTION IN HOMO- AND HETEROZYGOTES

The nineteen HLA-B*48 alleles can be combined in 190 homozygous and heterozygous combinations. Seventy-five of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products generated by primer mixes 5, 8 and 15 were not considered in these calculations.

++++-----	--+-----	4802,4807 = 4802,4816
+++-+----	---+--+--	4807,4814 = 4814,4816
+++-+----	---+-----	4801,4817 = 4803,4807 = 4803,4816 =
		4807,4817 = 4816,4817
+++-+----	---+--+--	4801,4814 = 4814,4818
+++--++--	+--+--+--	4805,4807 = 4805,4816
+++--++--	+--+--+--	4801,4805 = 4805,4815 = 4805,4819
+++-----	+--+-----	4807,4808 = 4808,4816
+++-----	+--+-----	4807,4819 = 4816,4819
+++-----	-++-+---	4807,4813 = 4813,4816
+++-----	-++-+---	4804,4807 = 4804,4816
+++-----	---+-----	4807,4809 = 4809,4816
+++-----	---+--+--	4807,4812 = 4812,4816
+++-----	---+--+--	4807,4810 = 4810,4816
+++-----	---+--+--	4807,4811 = 4811,4816
+++-----	---+--+--	4807,4818 = 4816,4818
+++-----	---+-----	4807,4815 = 4815,4816
+++-----	---+-----	4801,4807 = 4801,4816 = 4807,4807 =
		4807,4816
+++-----	+--+-----	4801,4808 = 4808,4815 = 4808,4819 =
		4815,4819
+++-----	+--+-----	4801,4819 = 4819,4819
+++-----	---+-----	4801,4809 = 4809,4809
+++-----	---+--+--	4801,4812 = 4810,4818 = 4812,4818
+++-----	---+--+--	4801,4811 = 4811,4811
+++-----	---+--+--	4801,4818 = 4818,4818
+++-----	---+-----	4801,4815 = 4815,4815
+++-+----	---+--+--	4812,4817 = 4814,4817
+++-+----	---+-----	4803,4817 = 4817,4817
+++-+----	---+--+--	4803,4812 = 4803,4814
+--+-----	-++-+---	4804,4810 = 4804,4813
+--+-----	---+--+--	4810,4814 = 4812,4814 = 4814,4814
+-----+--	+--+--+--	4805,4805 = 4805,4808 = 4805,4810
+-----+--	-++-+---	4810,4813 = 4813,4813
+-----+--	---+--+--	4810,4812 = 4812,4812

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SPECIFICITY TABLE

HLA-B*48 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-B*48 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*48 alleles ³	Other amplified HLA-B alleles ⁴
1	385 bp	800 bp	480101-4805, 4807-4815, 4817-4819	0817, 3803, 3804, 390201-390202, 3908, 391301-391302, 3922, 3923, 400101-400106, 4007, 4009-4011 [?] , 401401-4016 [?] , 4018 [?] , 4019 [?] , 4021 [?] , 4022N [?] , 4023, 4024 [?] , 4026-4038 [?] , 4040 [?] , 4042-4050 [?] , 4051, 4052-4058 [?] , 4059, 4060, 4061-4065 [?] , 4066, 4067 [?] , 4071 [?] , 407201-4073, 4074-4076 [?] , 4077, 4078 [?] , 4079, 4080-4088 [?] , 4090-4092 [?] , 4101-4108, 4418, 4501-4505, 4507-4509, 4901-4905, 5001, 5002, 5004
2^{5,6}	145 bp	800 bp	480101-480102, 480301-480302, 4806, 4807, 4809, 4811, 4815-4819	2736, 400101-400106, 4007, 4010, 4012, 4021-4023, 4025, 4030, 4031, 4033, 4034, 4036, 4038, 4042, 4043, 4045-4049, 4052, 4054, 4055, 4059-4061, 4063, 4066, 4067, 4069, 4073, 4074, 4076, 4079-4081, 4084, 4087, 4088, 4431, 4606, 8101-8104N
3	175 bp	800 bp	480101-480102, 4804, 4806, 4807, 4809, 4811, 4815, 4816, 4818, 4819	2724, 4031, 4045, 4080, 8101-8104N
4	150 bp	1070 bp	4802	1301, 1306, 1307N, 1312, 1313, 1317, 1320, 1322, 1502, 1513, 1520, 1521, 1525, 1536, 1544, 1562, 1577, 1580, 1585, 1588, 1589, 9506, 9512, 9521,

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				9539, 9544, 1822, 2719, 2730, 350101-350403, 3506-350803, 3510-3517, 3519-3521, 3523-3530, 3533-3536, 3538-3542, 3545-3550, 3552-3557, 3559, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3580-3585, 3590-3594, 370101-370104, 3703N-3706, 3708, 3710, 3711, 3713, 3942, 4028, 44020101-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-446402, 5104, 5142, 5146, 5156, 530101-5306, 5308-5313, 5315, 5316, 5514, 5609, 5611, 5612, 570101-5710, 5712, 5714-5717, 580101-580102, 5804, 5805, 5809-5815, 5817N, 5819, 8301
5⁷	230, 285 bp	1070 bp	480301-480302, 4814, 4817	0707, 0709, 0711, 071802, 9538, 3566, 400101-400106, 4007, 4010, 4012, 401401-401403, 4021-4023, 4025, 4033, 4036, 4038, 4042, 4043, 4046-4049, 4052, 4054, 4055, 4059-4063, 4065-4067, 4069, 407201-4074, 4076, 4077, 4079, 4081, 4084, 4087, 4088, 4108, 4431, 4606
6	170 bp	1070 bp	4805	
7	385 bp	1070 bp	4806	070201-0712, 0714-0753, 0755-0764, 0766, 080101-0809, 0811-0816, 0818-0836, 1401-1409, 380101-380202, 3805-3817, 39010101-39010102L, 390103-390105, 3903-3907, 3909-3912, 3914-3920, 3924-3945, 4201, 4202, 4204-4209, 670101-670102, 7301, 8102
8^{6,8}	85, 225 bp	1070 bp	4807, 4816, 4817	4036, 4048, 4077
9	215 bp	1070 bp	4805, 4808,	070201-0703, 070501-0706,

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			4819	0708-0711, 0713, 0715-0717, 0720-0724, 0727-0733, 0735-0742, 0744-0747, 0749N-0765, 0806, 0820, 1507, 1545, 1568, 9526 3566, 3707, 4015, 4016, 4032, 4612
10	240 bp	800 bp	4804, 4813	0753, 1301-1303, 1306-1309, 1311-1322, 1509, 1530, 1542, 1548, 1558, 1573, 1583, 1586, 1826, 2707, 2711, 2720, 2724, 2727, 2733, 2743, 350201-350202, 350401-350403, 3506, 350901-350902, 3512, 3518, 3522, 3531, 3544, 3559, 3560, 3581, 3583, 3588, 3712, 380101, 380201-3817, 39010101-39010102L, 390103-390602, 3908-3914, 3916, 3918-3920, 3922-3924, 3926-3928, 3930-3942, 3944, 3945, 400201-400203, 4004-400602, 4008, 4011, 4013, 4019, 4026-4029, 4035, 4037, 4039, 4040, 4050, 4051, 4053, 4056-4058, 4064, 4068, 4070, 4071, 4075, 4078, 4082, 4083, 4085, 4086, 4090-4092, 4106, 4208, 4410, 4414, 4462, 4611, 510101-5116, 5119-512402, 5126-5136, 5138-5141N, 5143, 5144N, 5146, 5148-5157, 520101-5211, 5213, 5214, 5401-5405N, 5407, 5408N, 5410-5413, 550101-5505, 5507-5513, 5515-5519, 5521, 5522, 5524-5526, 5528-5532, 5601, 5602, 5604-5608, 5610, 5612-5617, 5619N-5623, 5702-570302, 5707, 5709, 5712, 5717, 5808, 5901-5903 670101-6702, 7801-7806, 8201, 8202
11	170 bp	800 bp	480101-4805, 4807-4819	0727, 0750, 0804, 1503, 1547, 1554, 1561, 1562, 1564, 1568, 1569, 1591, 1598, 9503, 9523, 9527,

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				9531, 9532, 180101-1803, 1805, 1806, 1808-1815, 1817N-1828, 370101-3714, 3803, 390201-390202, 3908, 391301-391302, 3923, 3939, 4012
12^{5,6}	105 bp	1070 bp	4809	
13^{5,6,9}	130 bp	1070 bp	4805, 4810, 4812, 4813, 4814 ^{weakly}	070501-0706, 0732, 0734, 0740, 0753, 080101-0806, 0808N-0813, 0815, 0816, 0818-0827, 0829-0836, 1303, 1322, 1509, 1530, 1542, 1548, 1558, 1573, 1583, 1586, 1826, 2707, 2711, 2720, 2727, 2733, 2743, 350201-350202, 350401- 350403, 3506, 350901- 350902, 3512, 3518, 3522, 3531, 3544, 3559, 3560, 3581, 3583, 3587, 3588, 3712, 380101, 380201- 380202 ^{weakly} , 3803-3817, 39010101-39010102L, 390103-390602, 3908-3914, 3916, 3918-3920, 3922, 3924, 3926-3928, 3930-3941, 3945, 400201-400203, 4004- 400602, 4008, 4011, 4013- 4016, 4019, 4026-4029, 4035, 4037, 4039, 4040, 4050, 4053, 4056-4058, 4064, 4068, 4070, 4071, 407201- 407202 ^{weakly} , 4075, 4077 ^{weakly} , 4078, 4082, 4083, 4086, 4090, 4091 ^{weakly} , 4101- 410302, 4105-4108, 4201, 4202, 4204-4208, 4410, 4414, 4462, 4611, 510101 -5116, 5119-512402, 5126, 5128- 5136, 5138-5141N, 5143, 5144N, 5146, 5148-5157, 520101-5211, 5213, 5214, 5401-5405N, 5407, 5408N, 5410, 5412, 5413, 550101- 5505, 5507-5513, 5515-5522, 5524-5526, 5528-5532, 5601, 5602, 5604-5608, 5610, 5612- 5617, 5619N-5623, 5702-

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				570302, 5707, 5709, 5712, 5717, 5808, 5901-5903, 670101-6702, 7801-7806, 8201, 8202
14	250 bp	1070 bp	4811	
15^{5,6,10}	140, 435 bp	1070 bp	4812, 4814, 4818	0736, 0738, 0803, 1405, 1507, 1545, 1555, 1568, 9526, 1814, 2732, 3505, 3522, 3551, 3558, 3566, 3572, 380101-380102, 3805- 3807, 3809-3814, 3816, 3903, 3914, 3924, 3929, 3937, 4035, 4071, 4418, 4454, 4612, 4901, 4903-4905, 5314, 5818
16^{5,6}	125 bp	1070 bp	4805, 4808, 4815	070201-0724, 0726-0732, 0734-0741, 0743-0747, 0749N, 0750, 0752, 0754- 0759, 0761-0766, 0806, 0820, 9538, 3707, 4015, 4016, 4023, 4032

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*48 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 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*48 SSP subtyping.

In addition, wells number 2, 3, 10 and 11 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band.

In the presence of a specific amplification the intensity of the control band often decreases.

³The B*4806 and B*8102 alleles give rise to identical amplification patterns. These two alleles can be separated by e.g. the HLA-B low resolution SSP kit.

The B*4810 and B*0817 alleles give rise to identical amplification patterns. These two alleles can be separated by e.g. the HLA-B low resolution SSP kit.

The B*4813 and B*3803, 390201-390202, 3908 and 391301-391302 give rise to identical amplification patterns. These alleles can be separated by e.g. the HLA-B low resolution SSP kit.

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⁴Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B*48 alleles will be amplified by primer mixes 1 to 5 and 7 to 11, 13, 15 and 16

⁵Primer mixes 2, 12, 13, 15 and 16 have a tendency of giving rise to nonspecific amplifications.

⁶Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁷Primer mix 5: Specific PCR fragment of 230 bp in the B*4814 and the B*0709, 0711, 3566 and 4059 alleles. Specific PCR fragment of 285 bp in the B*480301-480302, 4817 and the B*0707, 071802, 400101-400106, 4007, 4010, 4012, 401401-401403, 4021-4023, 4025, 4033, 4036, 4042, 4043, 4046-4049, 4054, 4055, 4061-4063, 4065-4067, 4069, 407201-4074, 4076, 4077, 4079, 4081, 4084, 4087, 4088, 4108 and 4431 alleles. In the B*9538, 4038, 4052, 4060 and 4606 alleles both specific PCR fragments may be obtained.

⁸Primer mix 8: Specific PCR fragment of 85 bp in the B*4816 allele. Specific PCR fragment of 225 bp in the B*4807, 4817 and B*4036, 4048 and 4077 alleles.

⁹Primer mix 13 might faintly amplify the B*13xx alleles.

¹⁰Primer mix 15 has a tendency of giving rise to primer oligomer formation.

Primer mix 15: Specific PCR fragment of 140 bp in the B*4812, 4814 and B*1405, 1507, 1545, 1555, 1568, 9526, 1814, 2732, 3505, 3522, 3551, 3558, 3566, 3572, 3903, 3914, 3924, 3929, 3937, 4035, 4071, 4454, 4612, 5314 and 5818 alleles. Specific PCR fragment of 435 bp in the B*4818 and B*0736, 0738, 0803, 380101-380102, 3805-3807, 3809-3814, 3816, 4418, 4901 and 4903-4905 alleles.

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

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INTERPRETATION TABLE								
HLA-B*48 SSP subtyping								
Amplification patterns of the B*4801 to 4819 alleles								
	Well⁴							
	1	2	3	4	5	6	7	8
Length of spec.	385	145	175	150	230	170	385	85
PCR product(s)					285			225
Length of int.	800	800	800	1070	1070	1070	1070	1070
pos. control¹								
5'-primer(s)²	44	499	363	355	363	144	44	260
	5'-ggC ^{3'}	5'-TCT ^{3'}	5'-AgC ^{3'}	5'-TCA ^{3'}	5'-Agg ^{3'}	5'-TCC ^{3'}	5'-ggC ^{3'}	5'-ggT ^{3'}
					419			419
					5'-gTC ^{3'}			5'-gTT ^{3'}
3'-primer(s)³	259	605	499	463	605	272	259	302
	5'CTC ^{3'}	5'-gCT ^{3'}	5'-ggA ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	5'-Tgg ^{3'}	5'-gTT ^{3'}	5'-ggC ^{3'}
								605
								5'-gCT ^{3'}
Well No.	1	2	3	4	5	6	7	8
HLA-B allele								
*480101-480102	1	2	3					
*4802	1			4				
*480301-480302	1	2			5			
*4804	1		3					
*4805	1					6		
*4806, 8102⁵		2	3				7	
*4807	1	2	3					8
*4808	1							
*4809	1	2	3					
*4810, 0817⁶	1							
*4811	1	2	3					
*4812	1							
*4813, 3803, 390201-390202, 3908, 391301-391302⁷	1							
*4814	1				5			
*4815	1	2	3					
*4816		2	3					8
*4817	1	2			5			8
*4818	1	2	3					
*4819	1	2	3					
Well No.	1	2	3	4	5	6	7	8

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INTERPRETATION TABLE								
HLA-B*48 SSP subtyping								
Amplification patterns of the B*4801 to 4819 alleles								
Well ⁴								
9	10	11	12	13	14	15	16	
215	240	170	105	130	250	140	125	Length of spec. PCR product(s)
						435		
1070	800	800	1070	1070	1070	1070	1070	Length of int. pos. control ¹
363	412	142	693	412	363	44	527	5'-primer(s) ²
5'-AgC ^{3'}	5'-ATA ^{3'}	5'-TCT ^{3'}	5'-ggT ^{3'}	5'-ATA ^{3'}	5'-AgC ^{3'}	5'-ggC ^{3'}	5'-TgA ^{3'}	
						363		
						5'-AgC ^{3'}		
539	610	272	755	499	572	309	610	3'-primer(s) ³
5'-TCC ^{3'}	5'-CTg ^{3'}	5'-Tgg ^{3'}	5'-CAA ^{3'}	5'-ggT ^{3'}	5'-gCA ^{3'}	5'-ATC ^{3'}	5'-CTC ^{3'}	
						463		
						5'-gCT ^{3'}		
9	10	11	12	13	14	15	16	Well No.
								HLA-B allele
		11						*480101-480102
		11						*4802
		11						*480301-480302
	10	11						*4804
9		11		13			16	*4805
		11						*4806, 8102 ⁵
		11						*4807
9		11					16	*4808
		11	12					*4809
		11		13				*4810, 0817 ⁶
		11			14			*4811
		11		13		15		*4812
	10	11		13				*4813, 3803, 390201-390202, 3908, 391301-391302 ⁷
		11		w		15		*4814
		11					16	*4815
		11						*4816
		11						*4817
		11				15		*4818
9		11						*4819
9	10	11	12	13	14	15	16	Well No.

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Length of spec.	385	145	175	150	230	170	385	85
PCR product(s)					285			225
Well No.	1	2	3	4	5	6	7	8
*070201-0703, 0708, 0710, 0715-0717, 0720-0724, 0728-0731, 0735, 0737, 0739, 0741, 0744-0747, 0749N, 0752, 0755-0759, 0761-0764							7	
*0704, 0712, 0714, 071801, 0719, 0726, 0743, 0766							7	
*070501-0706, 0732, 0740, 0806, 0820							7	
*0707, 071802					5		7	
*0709, 0711					5		7	
*0713, 0754, 0765								
*0725, 0748, 0807, 0814, 0828, 1401-1404, 140601-1409, 3907, 3915, 3917, 3925N, 3943, 4209, 7301							7	
*0727, 0750							7	
*0733, 0742, 0751, 0760							7	
*0734							7	
*0736, 0738							7	
*0753							7	
*080101-0802, 0805, 0808N, 0809, 0811-0813, 0815, 0816, 0818, 0819N, 0821-0827, 0829-0836, 4201, 4202, 4204-4207							7	
*0803							7	
*0804							7	
*0810, 3587, 5520								
*1301, 1306, 1307N, 1312, 1313, 1317, 1320				4				
*130201-130204, 1308Q, 1309, 1311, 1314-1316, 1318, 1319, 1321, 5127N, 5411								
Well No.	1	2	3	4	5	6	7	8

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215	240	170	105	130	250	140	125	Length of spec. PCR product(s)
9	10	11	12	13	14	15	16	Well No.
						435		
9							16	*070201-0703, 0708, 0710, 0715-0717, 0720-0724, 0728-0731, 0735, 0737, 0739, 0741, 0744-0747, 0749N, 0752, 0755-0759, 0761-0764
							16	*0704, 0712, 0714, 071801, 0719, 0726, 0743, 0766
9				13			16	*070501-0706, 0732, 0740, 0806, 0820
							16	*0707, 071802
9							16	*0709, 0711
9							16	*0713, 0754, 0765
								*0725, 0748, 0807, 0814, 0828, 1401- 1404, 140601-1409, 3907, 3915, 3917, 3925N, 3943, 4209, 7301
9		11					16	*0727, 0750
9								*0733, 0742, 0751, 0760
				13			16	*0734
9						15	16	*0736, 0738
9	10			13				*0753
				13				*080101-0802, 0805, 0808N, 0809, 0811 0813, 0815, 0816, 0818, 0819N, 0821- 0827, 0829-0836, 4201, 4202, 4204- 4207
				13		15		*0803
		11		13				*0804
				13				*0810, 3587, 5520
	10							*1301, 1306, 1307N, 1312, 1313, 1317, 1320
	10							*130201-130204, 1308Q, 1309, 1311, 1314-1316, 1318, 1319, 1321, 5127N, 5411
9	10	11	12	13	14	15	16	Well No.

Lot No.: **03F**

Lot-specific information

Length of spec.	385	145	175	150	230	170	385	85
PCR product(s)					285			225
Well No.	1	2	3	4	5	6	7	8
*1303, 1509, 1530, 1542, 1548, 1558, 1573, 1583, 1586, 2707, 2711, 2720, 2727, 2733, 2743, 350901-350902, 3518, 3531, 3544, 3560, 3588, 400201-400203, 4004-400602, 4008, 4013, 4039, 4068, 4070, 4611, 510101-5103, 5105-5116, 5119-512402, 5126, 5128-5136, 5138-5141N, 5143, 5144N, 5148-5155, 5157, 520101-5211, 5213, 5214, 5401-5405N, 5407, 5408N, 5410, 5412, 5413, 550101-5505, 5507-5513, 5515-5519, 5521, 5522, 5524-5526, 5528-5532, 5601, 5602, 5604-5608, 5610, 5613-5617, 5619N-5623, 5808, 5901-5903, 6702, 7801-7806, 8201, 8202								
*1322, 350201-350202, 350401-350403, 3506, 3512, 3559, 3581, 3583, 4410, 4414, 4462, 5104, 5146, 5156, 5612, 5702-570302, 5707, 5709, 5712, 5717				4				
*1405, 380102, 3929							7	
*1502, 1513, 1520, 1521, 1525, 1536, 1544, 1577, 1580, 1585, 1588, 1589, 9506, 9512, 9521, 9539, 9544, 2719, 2730, 350101-350107, 3503, 3507-350803, 3510, 3511, 3513-3517, 3519-3521, 3523-3530, 3533-3536, 3538-3542, 3545-3550, 3552-3557, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3580, 3582, 3584, 3585, 3590-3594, 44020101-4409, 4411-4413, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-4461N, 4463-446402, 5142, 530101-5306, 5308-5313, 5315, 5316, 5514, 5609, 5611, 570101-570103, 5704-5706, 5708, 5710, 5714-5716, 580101-580102, 5804, 5805, 5809-5815, 5817N, 5819, 8301				4				
Well No.	1	2	3	4	5	6	7	8

Lot No.: **03F**

Lot-specific information

215	240	170	105	130	250	140	125	Length of spec. PCR product(s)
9	10	11	12	13	14	15	16	Well No.
	10			13				*1303, 1509, 1530, 1542, 1548, 1558, 1573, 1583, 1586, 2707, 2711, 2720, 2727, 2733, 2743, 350901-350902, 3518, 3531, 3544, 3560, 3588, 400201-400203, 4004-400602, 4008, 4013, 4039, 4068, 4070, 4611, 510101-5103, 5105-5116, 5119-512402, 5126, 5128-5136, 5138-5141N, 5143, 5144N, 5148-5155, 5157, 520101-5211, 5213, 5214, 5401-5405N, 5407, 5408N, 5410, 5412, 5413, 550101-5505, 5507-5513, 5515-5519, 5521, 5522, 5524-5526, 5528-5532, 5601, 5602, 5604-5608, 5610, 5613-5617, 5619N-5623, 5808, 5901-5903, 6702, 7801-7806, 8201, 8202
	10			13				*1322, 350201-350202, 350401-350403, 3506, 3512, 3559, 3581, 3583, 4410, 4414, 4462, 5104, 5146, 5156, 5612, 5702-570302, 5707, 5709, 5712, 5717
						15		*1405, 380102, 3929
								*1502, 1513, 1520, 1521, 1525, 1536, 1544, 1577, 1580, 1585, 1588, 1589, 9506, 9512, 9521, 9539, 9544, 2719, 2730, 350101-350107, 3503, 3507-350803, 3510, 3511, 3513-3517, 3519-3521, 3523-3530, 3533-3536, 3538-3542, 3545-3550, 3552-3557, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3580, 3582, 3584, 3585, 3590-3594, 44020101-4409, 4411-4413, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-4461N, 4463-446402, 5142, 530101-5306, 5308-5313, 5315, 5316, 5514, 5609, 5611, 570101-570103, 5704-5706, 5708, 5710, 5714-5716, 580101-580102, 5804, 5805, 5809-5815, 5817N, 5819, 8301
9	10	11	12	13	14	15	16	Well No.

Lot No.: **03F**

Lot-specific information

Length of spec.	385	145	175	150	230	170	385	85
PCR product(s)					285			225
Well No.	1	2	3	4	5	6	7	8
*1503, 1547, 1554, 1561, 1564, 1569, 1591, 1598, 9503, 9523, 9527, 9531, 9532, 180101-1803, 1805, 1806, 1808-1813, 1815, 1817N-1821, 1823N-1825, 1827, 1828, 3702, 3709, 3714								
*1507, 1545, 9526, 4612								
*1555, 2732, 3505, 3551, 3558, 3572, 4454, 5314, 5818								
*1562, 1822, 370101-370104, 3703N-3706, 3708, 3710, 3711, 3713				4				
*1568								
*9538					5			
*1814								
*1826, 3712								
*2724			3					
*2736		2						
*3522								
*3566					5			
*3707								
*380101, 3805-3807, 3809-3814, 3816, 3903, 3914, 3924, 3937							7	
*3808, 3815, 3817, 39010101-39010102L, 390103-390105, 3904-390602, 3909-3912, 3916, 3918-3920, 3926-3928, 3930-3936, 3938Q, 3940N, 3941, 3945, 4208, 670101-670102							7	
*380201-380202							7	
*3804, 3922, 4106	1							
*3923	1							
*3939							7	
*3942				4			7	
*3944							7	
*400101-400106, 4007, 4059, 4060, 4066, 4073, 4079	1	2			5			
*4010, 4021, 4022N, 4033, 4038, 4042, 4043, 4046, 4047, 4049, 4052, 4054, 4055, 4061, 4063, 4067, 4074, 4076, 4081, 4084, 4087, 4088	?	2			5			
*4009, 4018, 4024, 4044	?							
Well No.	1	2	3	4	5	6	7	8

Lot No.: **03F**

Lot-specific information

215	240	170	105	130	250	140	125	Length of spec.
						435		PCR product(s)
9	10	11	12	13	14	15	16	Well No.
		11						*1503, 1547, 1554, 1561, 1564, 1569, 1591, 1598, 9503, 9523, 9527, 9531, 9532, 180101-1803, 1805, 1806, 1808-1813, 1815, 1817N-1821, 1823N-1825, 1827, 1828, 3702, 3709, 3714
9						15		*1507, 1545, 9526, 4612
						15		*1555, 2732, 3505, 3551, 3558, 3572, 4454, 5314, 5818
		11						*1562, 1822, 370101-370104, 3703N-3706, 3708, 3710, 3711, 3713
9		11				15		*1568
							16	*9538
		11				15		*1814
	10	11		13				*1826, 3712
	10							*2724
								*2736
	10			13		15		*3522
9						15		*3566
9		11					16	*3707
	10			13		15		*380101, 3805-3807, 3809-3814, 3816, 3903, 3914, 3924, 3937
	10			13				*3808, 3815, 3817, 39010101-39010102L, 390103-390105, 3904-390602, 3909-3912, 3916, 3918-3920, 3926-3928, 3930-3936, 3938Q, 3940N, 3941, 3945, 4208, 670101-670102
	10			w				*380201-380202
	10			13				*3804, 3922, 4106
	10	11						*3923
	10	11		13				*3939
	10			13				*3942
	10							*3944
								*400101-400106, 4007, 4059, 4060, 4066, 4073, 4079
								*4010, 4021, 4022N, 4033, 4038, 4042, 4043, 4046, 4047, 4049, 4052, 4054, 4055, 4061, 4063, 4067, 4074, 4076, 4081, 4084, 4087, 4088
								*4009, 4018, 4024, 4044
9	10	11	12	13	14	15	16	Well No.

Lot No.: **03F**

Lot-specific information

Length of spec.	385	145	175	150	230	170	385	85
PCR product(s)					285			225
Well No.	1	2	3	4	5	6	7	8
*4011, 4019, 4026, 4027, 4029, 4037, 4040, 4050, 4053, 4056-4058, 4064, 4075, 4078, 4082, 4083, 4086, 4090	?							
*4012		2			5			
*401401-401403	?				5			
*4015, 4016	?							
*4023	1	2			5			
*4025, 4069, 4431, 4606		2			5			
*4028	?			4				
*4030, 4034	?	2						
*4031, 4045, 4080	?	2	3					
*4032	?							
*4035, 4071	?							
*4036, 4048	?	2			5			8
*4051	1							
*4062, 4065	?				5			
*407201-407202	1				5			
*4077	1				5			8
*4085, 4092	?							
*4091	?							
*4101-410302, 4105, 4107	1							
*4104, 4501-4505, 4507-4509, 4902, 5001, 5002, 5004	1							
*4108	1				5			
*4418, 4901, 4903-4905	1							
*8101, 8103, 8104N		2	3					
HLA-B allele								
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*48 SSP subtyping.

In addition, wells number 2, 3, 10 and 11 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band.

²The nucleotide position, in the 1st, 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, 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.: **03F**

Lot-specific information

215	240	170	105	130	250	140	125	Length of spec.
						435		PCR product(s)
9	10	11	12	13	14	15	16	Well No.
	10			13				*4011, 4019, 4026, 4027, 4029, 4037, 4040, 4050, 4053, 4056-4058, 4064, 4075, 4078, 4082, 4083, 4086, 4090
		11						*4012
				13				*401401-401403
9				13			16	*4015, 4016
							16	*4023
								*4025, 4069, 4431, 4606
	10			13				*4028
								*4030, 4034
								*4031, 4045, 4080
9							16	*4032
	10			13		15		*4035, 4071
								*4036, 4048
	10							*4051
								*4062, 4065
				w				*407201-407202
				w				*4077
	10							*4085, 4092
	10			w				*4091
				13				*4101-410302, 4105, 4107
								*4104, 4501-4505, 4507-4509, 4902, 5001, 5002, 5004
				13				*4108
						15		*4418, 4901, 4903-4905
								*8101, 8103, 8104N
								HLA-B allele
9	10	11	12	13	14	15	16	Well No.

⁴Primer mix 5: Specific PCR fragment of 230 bp in the B*4814 and the B*0709, 0711, 3566 and 4059 alleles. Specific PCR fragment of 285 bp in the B*480301-480302, 4817 and the B*0707, 071802, 400101-400106, 4007, 4010, 4012, 401401-401403, 4021-4023, 4025, 4033, 4036, 4042, 4043, 4046-4049, 4054, 4055, 4061-4063, 4065-4067, 4069, 407201-4074, 4076, 4077, 4079, 4081, 4084, 4087, 4088, 4108 and 4431 alleles. In the B*9538, 4038, 4052, 4060 and 4606 alleles both specific PCR fragments may be obtained.

Primer mix 8: Specific PCR fragment of 85 bp in the B*4816 allele. Specific PCR fragment of 225 bp in the B*4807, 4817 and B*4036, 4048 and 4077 alleles.

Primer mix 15: Specific PCR fragment of 140 bp in the B*4812, 4814 and B*1405, 1507, 1545, 1555, 1568, 9526, 1814, 2732, 3505, 3522, 3551, 3558, 3566, 3572, 3903, 3914, 3924, 3929, 3937, 4035, 4071, 4454, 4612, 5314 and 5818 alleles. Specific PCR fragment of 435 bp in the B*4818 and B*0736, 0738, 0803, 380101-380102, 3805-3807, 3809-3814, 3816, 4418, 4901 and 4903-4905 alleles.

Lot No.: **03F**

Lot-specific information

⁵The B*4806 and B*8102 alleles give rise to identical amplification patterns. These two alleles can be separated by e.g. the HLA-B low resolution SSP kit.

⁶The B*4810 and B*0817 alleles give rise to identical amplification patterns. These two alleles can be separated by e.g. the HLA-B low resolution SSP kit.

⁷The B*4813 and B*3803, 390201-390202, 3908 and 391301-391302 give rise to identical amplification patterns. These alleles can be separated by e.g. the HLA-B low resolution SSP kit.

'w', might be weakly amplified.

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

Lot No.: **03F**

Lot-specific information

CELL LINE VALIDATION SHEET																					
HLA-B*48 SSP primer set																					
				Prod. No.:	Well																
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	15	
					200310701	200620602	200733603	200310704	200850605	200310706	200310707	200850608	2.0041E+10	2.0041E+10	2.0041E+10	200620612	200733613	200511314	200850615	200850616	
	IHC cell line	B*																			
1	9001 SA	*0702			-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+
2	9280 LK707	*5201	*7301		-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-
3	9011 E4181324	*52011			-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-
4	9275 GU373	*1510	*5301		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*3701			-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
6	9353 SM	*3901	*5101		-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-
7	9020 QBL	*1801			-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
8	9007 DEM	*5701			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*3801			-	-	-	-	-	-	+	-	-	+	-	-	+	-	+	-	-
10	9107 LKT3	*5401			-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-
11	9051 PITOUT	*4403			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*5701			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9067 BTB	*2705			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*1501	*1520		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*4001			+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*4002			-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-
17	9008 WILJON	*1801			-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
18	9257 32367	*1401	*5601		-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-
19	9038 BM16	*1801			-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
20	9059 SLE005	*4001			+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*1501			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*3503			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*4002	*5602		-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-
24	9035 JBUSH	*3801			-	-	-	-	-	-	+	-	-	+	-	-	+	-	+	-	-
25	9049 IBW9	*1402			-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*5801			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*0705	*5101		-	-	-	-	-	-	+	-	+	+	-	-	+	-	-	-	-
28	9320 BEL5GB	*4402	*4403		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*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		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-

Lot No.: **03F**

Lot-specific information

CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-B*48 SSP

Product number: 101.546-06u – without *Taq* polymerase
Lot number: 03F
Expiry date: 2010-July-01
Number of tests: 6
Number of wells per test: 16

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2003-107-01	9	2004-125-09
2	2006-206-02	10	2004-125-10
3	2007-336-03	11	2004-125-11
4	2003-107-04	12	2006-206-12
5	2008-506-05	13	2007-336-13
6	2003-107-06	14	2005-113-14
7	2003-107-07	15	2008-506-15
8	2008-506-08	16	2008-506-16

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

No DNAs carrying the alleles to be amplified by primer solutions 6, 8, 12 and 14 were available. The specificities of the primers in primer solution 8 were tested by separately adding one additional 3'-primer, respectively one additional 5'-primer. In primer solution 6 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solutions 12 and 14 it was only possible to test the 5'-primer, the 3'-primer was not possible to test.

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

Date of approval: 2009-May-27

Approved by:

Quality Control, Supervisor

Lot No.: **03F**

Lot-specific information

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*48
Product number: 101.546-06u
Lot number: 03F

Intended use: HLA-B*48 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2000 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, conformity assessed using Annex IV, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom. (Notified Body number: 0088.)

Saltsjöbaden, Sweden
2009-May-27

Olle Olerup
Managing Director

Lot No.: **03F**

Lot-specific information

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