

Olerup SSP[®] HLA-B*57

Product number:	101.567-12 – including <i>Taq</i> polymerase
Lot number:	66G
Expiry date:	2012-February-01
Number of tests:	12
Number of wells per test:	21
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

This Product Description is only valid for Lot No. 66G.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] HLA-B*57 LOT

The HLA-B*57 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP*[®] HLA-B*57 lot was made (Lot No. 59F).

Five wells have been added to the HLA-B*57 kit, wells
17 to 21.

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

Well	5'-primer	3'-primer	rationale
15	Added	Added	New primer pair for the B*5729 allele.
17	New	New	New primer pair for the B*5722 and B*5727 alleles.
18	New	New	New primer pair for the B*5723 allele.
19	New	New	New primer pair for the B*5725 allele.
20	New	New	New primer pair for the B*5720 and B*5726 alleles.
21	New	New	New primer pair for the B*5721 allele.

PRODUCT DESCRIPTION

HLA-B*57 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*5701 to B*5729 alleles.

PLATE LAYOUT

Each test consists of 21 PCR reactions in a 24 well cut PCR plate. Wells 22 to 24 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	empty	empty	empty

The 24 well cut PCR plate is marked with ‘HLA-B*57’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘66G’.

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

The PCR plates are heat-sealed with a PCR-compatible foil.

Please note: When removing each 24 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

The interpretation of HLA-B*57 SSP subtypings will be influenced by the B*07, four B*08, many B*13, the B*14, most B*15, six B*18, many B*27, most B*35, most B*37, two B*38, four B*39, several B*40, the B*4213, the B*44, the B*46, six B*48, the B*49, the B*50, the B*51, the B*52, the B*53, the B*5406, most B*55, several B*56, most B*58, most B*78 and the B*8301 allele when present on the other haplotype. In addition, the Cw*0132 and Cw*0620 alleles will be amplified by primer mix 15, the Cw*0510 allele will be weakly amplified and the Cw*1525 allele will be amplified by primer mix 4.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*57 alleles¹, i.e. **B*5701 to B*5729**, recognized by the HLA Nomenclature Committee in January 2010² will give rise to unique amplification patterns by the primers in the HLA-B*57 subtyping kit.

The HLA-B*57 subtyping kit cannot distinguish the B*570101 to B*570104 and the B*570106, the B*570201 and B*570202 or the B*570301 and B*570302 alleles.

¹The sequence of the B*5728N allele is not yet available

²HLA-B alleles listed on the IMGT/HLA web page 2010-January-15, release 2.28.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 28 HLA-B*57 alleles will give rise to 29 amplification patterns, that can be combined in 435 homozygous and heterozygous combinations. 208 of these genotypes do not give rise to unique amplification patterns. The different sizes of the specific PCR products generated by primer mixes 6, 8, 11, 14 to 16 and 20 were not considered in these calculations.

+++++---+ ---+--- -	5702, 5716 = 5707, 5719
+++++--- -+--- -	5702, 5710 = 5717, 5719
+++++--- -+---+ -	5712, 5720 = 5712, 5726
+++++--- -+---+ -	5701, 5712 = 570105, 5712 = 5702, 5718 = 5712, 5718
+++++--- -+--- -	5702, 5720 = 5702, 5726
+++++--- -+--- -	5701, 5702 = 570105, 5702 = 5703, 5719
+++++--- -+--- -	5707, 5714 = 5707, 5724
+++++--- -+--- -	5707, 5710 = 5716, 5717
+++++--- -+--- -	5707, 5720 = 5707, 5726
+++++--- -+--- -	5701, 5707 = 570105, 5707 = 5703, 5716 = 5707, 5716
+++++--- +---+ -	5709, 5722 = 5709, 5727
+++++--- +---+ -	5709, 5720 = 5709, 5726
+++++--- +---+ -	5701, 5709 = 570105, 5709 = 5709, 5714
+++++--- -+--- -	5714, 5717 = 5717, 5724
+++++--- -+--- -	5703, 5714 = 5703, 5724
+++++--- -+--- -	5717, 5720 = 5717, 5726
+++++--- -+--- -	5701, 5717 = 570105, 5717 = 5703, 5710 = 5710, 5717
+++++--- -+--- -	5703, 5720 = 5703, 5726
+++++--- -+--- -	5701, 5703 = 570105, 5703
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+++++--- -+--- -	5701, 5705 = 570105, 5705
+++-+- -+--- -	5704, 5720 = 5704, 5726
+++-+- -+--- -	5701, 5704 = 570105, 5704 = 5704, 5706 = 5706, 5719
+++-+- +---+ -	5713, 5720 = 5713, 5726
+++-+- +---+ -	5701, 5713 = 5713, 5722 = 5713, 5725 = 5713, 5727
+++-+- -+--- -	5719, 5720 = 5719, 5726
+++-+- -+--- -	5701, 5719 = 570105, 5719
+++-+- -+--- -	5706, 5714 = 5706, 5724
+++-+- -+--- -	5706, 5711 = 5706, 5729
+++-+- -+--- -	5706, 5720 = 5706, 5726
+++-+- -+--- -	5701, 5706 = 570105, 5706 = 5706, 5706
+++-+- -+--- -	5714, 5715 = 5715, 5724
+++-+- -+--- -	5711, 5715 = 5715, 5729
+++-+- -+--- -	5715, 5720 = 5715, 5726
+++-+- -+--- -	5701, 5715 = 570105, 5715 = 5715, 5715
+++-+- -+--- -	5714, 5716 = 5716, 5724
+++-+- -+--- -	5711, 5716 = 5716, 5729
+++-+- -+--- -	5701, 5716 = 570105, 5716
+++-+- +---+ -	5714, 5722 = 5722, 5724
+++-+- +---+ -	5711, 5722 = 5722, 5729
+++-+- +---+ -	5720, 5722 = 5722, 5726

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Lot-specific information

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+++----- +----+---- +-----	5701, 5722 = 570105, 5722 = 5722, 5722 = 5722, 5727
+++----- -+-+---- ------	5708, 5714 = 5708, 5724
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+++----- --+---+- +-----	5714, 5727 = 5724, 5727
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+++----- --+---+- --+--	5714, 5725 = 5724, 5725
+++----- --+---+- -+++-	5714, 5720 = 5714, 5726 = 5720, 5724 = 5724, 5726
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+++----- --+---+- ------	5701, 5714 = 5701, 5724 = 570105, 5714 = 570105, 5724 = 5714, 5714 = 5714, 5724
+++----- -----++- ------	5710, 5711 = 5710, 5729
+++----- -----++- -+++-	5710, 5720 = 5710, 5726
+++----- -----++- ------	5701, 5710 = 570105, 5710 = 5710, 5710
+++----- -----++- ------	5711, 5718 = 5718, 5729
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+++----- -----++- -+++-	5711, 5721 = 5721, 5729
+++----- -----++- ------	5701, 5711 = 5701, 5729 = 570105, 5729 = 5711, 5729 = 5729, 5729
+++----- -----++- -+++-	5718, 5720 = 5718, 5726
+++----- -----++- ------	5701, 5718 = 570105, 5718 = 5718, 5718
+++----- -----++- +---+	5720, 5727 = 5726, 5727
+++----- -----++- +-----	5701, 5727 = 570105, 5727 = 5727, 5727
+++----- -----++- -+++-	5720, 5723 = 5723, 5726
+++----- -----++- -+----	5701, 5723 = 570105, 5723 = 5723, 5723
+++----- -----++- --+--	5720, 5725 = 5725, 5726
+++----- -----++- --+--	5701, 5725 = 570105, 5725 = 5725, 5725
+++----- -----++- -+++-	5720, 5721 = 5721, 5726
+++----- -----++- -+++-	5701, 5720 = 5701, 5726 = 570105, 5720 = 570105, 5726 = 5720, 5726 = 5726, 5726
+++----- -----++- -+++-	5701, 5721 = 570105, 5721 = 5721, 5721
+++----- -----++- ------	5701, 5701 = 5701, 570105
+--+----+ ----+---- ------	5703, 5707 = 5705, 5707 = 5707, 5707
+--+----+ ----+---- ------	5703, 5717 = 5705, 5717 = 5717, 5717
+--+----+ ----+---- ------	5703, 5703 = 5703, 5705
+--+----+ ----+----+ ------	5702, 5712 = 5705, 5712 = 5712, 5712
+--+----+ ----+---- ------	5702, 5702 = 5702, 5705
+--+----+ +----+---- ------	5705, 5709 = 5709, 5709

5701 = 570101-570104 and 570106

5702 = 570201 and 570202

5703 = 570301 and 570302

SPECIFICITY TABLE

HLA-B*57 SSP subtyping

Specificities and sizes of the PCR products of the 21 primer mixes used for HLA-B*57 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*57 alleles	Other amplified HLA Class I alleles ³
1	150 bp	800 bp	*570101-570104, 570106-5710, 5712, 5714-5727, 5729	*0849, 130101-130103, 1306, 1307N, 1312, 1313, 1317, 1320, 132201-1323, 1325, 1326, 1328, 1329, 1336, 1410, 150201-150204, 1513, 1520, 1521, 152501, 152502, 1536, 1544, 1562, 1577, 1580, 1585, 1588, 1589, 9506, 9512, 9521, 9539, 9544, 9554, 9565, 9570, 1822, 2719, 2730, 350101-350403, 3506-350804, 3510-3517, 3519-3521, 3523-3530, 3533-3536, 3538-3542, 3545-3550, 3552, 3554-3557, 3559, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3580-3585, 3590-3596, 3598, 370101-370107, 3703N-3706, 3708, 3710, 3711, 3713-3721, 3820, 3942, 4028, 44020101-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-446402, 4466-4498, 480201, 480202, 5104, 5142, 5146, 5156, 530101-5313, 5315-5321, 5514, 5609, 5611, 5612, 580101, 580102, 580104, 5804, 5805, 5809-5815, 5817N, 5819, 5821-5824, 5828, 8301
2 ⁴	100 bp	1070 bp	*570101-570106, 5706, 5708, 5710, 5713-5716, 5718-5727, 5729	*5514, 5814
3	220 bp	1070 bp	*570101-570106, 570301,	*4030, 4034, 5514, 5814

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			570302, 5706-5708, 5710, 5714- 5718, 5720- 5723, 5725- 5727, 5729	
4⁴	100 bp	800 bp	*570201- 570302, 5705, 5707, 5709, 5712, 5717	*1313, 1321, 350201-350204, 350401-350403, 3506 ^w , 350901, 350902, 351201, 351202, 3518, 3559 ^w , 3581, 3583, 3588, 3595, 370101 ^w -370107 ^w , 3703N ^w -3706 ^w , 3708 ^w , 3710 ^w , 3711 ^w , 3712, 3713 ^w - 3721 ^w , 3820 ^w , 3942 ^w , 4004, 4028, 4030, 4034, 4064, 4068, 4099, 4213, 4462, 4477, 4482, 4817 ^w , 5104, 5146, 5156, 5319, 5612, 5828, Cw*0510^w, Cw*1524
5	220 bp	1070 bp	*570201, 570202, 5704, 5712, 5713, 5719	
6^{4,6}	95 bp, 180 bp	1070 bp	*5704, 5706	
7⁴	105 bp	1070 bp	*5715	
8^{4,7}	90 bp, 250 bp	1070 bp	*5707, 5716	
9⁵	170 bp	1070 bp	*5709, 5713, 5722	*070201-0724, 0726-0747, 0749N, 0750, 0752-0799, 0806, 0820, 0853, 1316, 1320, 1331, 1401- 1418, 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, 9581N-9584, 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, 490101-4909, 500101-5002, 5004-5009, 510101-5104, 5106, 5107, 5111N-5114, 5116-5118, 5121-512403, 5126-5130, 5132-5139, 5141N, 5143, 5145, 5146, 5148-5152, 5155-5172, 5174-5180, 5182-5189, 520101-5202, 5204-5209, 5211-5218, 5306, 530801, 530802, 5406, 550101-550106, 5503, 5505, 5509, 5511, 5514, 5515, 5517, 5521, 5524, 5525, 5528, 5529, 5531, 5533, 5536, 5538, 5603, 560501-5606, 5621, 5625, 5806, 5808, 5819, 7801-7803, 7805, 7806	
10	210 bp	1070 bp	*5708	
11⁸	165 bp, 215 bp	800 bp	*5709, *5514, 5814 5714, 5724	
12⁴	90 bp	1070 bp	*570201- *4030, 4034 570302, 5707, 5709, 5712, 5717	
13⁴	90 bp	800 bp	*570101- 5715, 5717- 5719, 5721- 5727, 5729	
14⁹	135 bp, 195 bp	1070 bp	*5710, 5717	
15^{4,5,10}	110 bp, 145 bp	1070 bp	*5711, 5729 *1401-1404, 1407N, 1409, 1411, 1412, 1414-1418, 1844, 5802, 5806, 5807, 5825, Cw*0132, Cw*0620	
16^{4,11}	85 bp, 160 bp	1070 bp	*5712, 5718	
17	210 bp	1070 bp	*5713, *5514 5722, 5727	
18	165 bp	1070 bp	*5723	
19	240 bp	1070 bp	*5713, 5725 *4030, 4034	
20^{4,12}	90 bp, 240 bp	1070 bp	*5720, 5726 *5514	
21	150 bp	1070 bp	*5721	

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*57 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*57 subtyping.

In addition, wells number 4, 11 and 13 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

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

³Due to the sharing of sequence motifs between HLA-B alleles some non-HLA-B*57 alleles will be amplified by primer mixes 1, 2, 3, 4, 9, 11, 12, 15, 17, 19 and 20. In addition, the Cw*0132 and Cw*0620 alleles will be amplified by primer mix 15, the Cw*0510 allele will be weakly amplified and the Cw*1525 allele will be amplified by primer mix 4.

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

⁵Primer mixes 9 and 15 have a tendency of giving rise to nonspecific amplifications.

⁶Primer mix 6: Specific PCR fragment of 95 bp in the B*5704 allele. Specific PCR fragment of 180 bp in the B*5706 allele.

⁷Primer mix 8: Specific PCR fragment of 90 bp in the B*5716 allele. Specific PCR fragment of 250 bp in the B*5707 allele.

⁸Primer mix 11: Specific PCR fragment of 165 bp in the B*5714 and B*5514 and 5814 alleles. Specific PCR fragment of 215 bp in the B*5709 and 5724 alleles.

⁹Primer mix 14: Specific PCR fragment of 135 bp in the B*5717 allele. Specific PCR fragment of 195 bp in the B*5710 allele.

¹⁰Primer mix 15: Specific PCR fragment of 110 bp in the B*5729 allele. Specific PCR fragment of 145 bp in the B*5711, 1401-1404, 1407N, 1409, 1411, 1412, 1414-1418, 1844, 5802, 5806, 5807, 5825, Cw*0132, Cw*0620 alleles.

¹¹Primer mix 16: Specific PCR fragment of 85 bp in the B*5712 allele. Specific PCR fragment of 160 bp in the B*5718 allele.

¹²Primer mix 20: Specific PCR fragment of 90 bp in the B*5720 allele. Specific PCR fragment of 240 bp in the B*5726 and the B*5514 alleles.

‘w’, might be weakly amplified.

INTERPRETATION TABLE													
HLA-B*57 SSP subtyping													
Amplification patterns of the B*5701 to 5729 alleles													
	Well ⁵												
	1	2	3	4	5	6	7	8	9	10	11	12	
Length of spec.	150	100	220	100	220	95	105	90	170	210	165	90	
PCR product						180		250			215		
Length of int. pos. control ¹	800	1070	1070	800	1070	1070	1070	1070	1070	1070	800	1070	
5'-primer(s) ²	355	362	362	355	362	362	209	209	527	320	362	362	
	5'-TCA ^{3'}	5'-ggT ^{3'}	5'-ggT ^{3'}	5'-TCA ^{3'}	5'-ggT ^{3'}	5'-ggT ^{3'}	5'-ggC ^{3'}	5'-ggA ^{3'}		5'-TgA ^{3'}	5'-CCC ^{3'}	5'-ggT ^{3'}	5'-ggT ^{3'}
								362					
								5'-ggT ^{3'}					
3'-primer(s) ³	463	419	539	412	539	418	271	256	3 rd	2 nd	486	412	
	5'-gCT ^{3'}	5'-Cgg ^{3'}	5'-TCA ^{3'}	5'-gTT ^{3'}	5'-TCC ^{3'}	5'-gTC ^{3'}	5'-CAC ^{3'}	5'-CCC ^{3'}	5'-TAT ^{3'}	5'-TCg ^{3'}	5'-gCg ^{3'}	5'-gTT ^{3'}	
		419				500		572			538		
		5'-Cag ^{3'}				5'-ggA ^{3'}		5'-gCg ^{3'}			5'-gTC ^{3'}		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	
HLA-B allele ⁴													
*570101-570104, 570106	1	2	3										
*570105		2	3										
*570201, 570202	1			4	5								12
*570301, 570302	1		3	4									12
*5704	1				5	6							
*5705	1			4									
*5706	1	2	3			6							
*5707	1		3	4				8					12
*5708	1	2	3							10			
*5709	1			4					9		11	12	
*5710	1	2	3										
*5711													
*5712	1			4	5								12
*5713		2			5				9				
*5714	1	2	3								11		
*5715	1	2	3				7						
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	

INTERPRETATION TABLE										
HLA-B*57 SSP subtyping										
Amplification patterns of the B*5701 to 5729 alleles										
Well⁵										
13	14	15	16	17	18	19	20	21		
90	135	110	85	210	165	240	90	150	Length of spec. PCR product	
	195	145	160				240		Length of int. pos. control ¹	
800	1070	1070	1070	1070	1070	1070	1070	1070		
209	103	361	256	362	130	362	209	352	5'-primer(s) ²	
5'-ggC ^{3'}	5'-CCT ^{3'}	5'-AgT ^{3'}	5'-ACg ^{3'}	5'-ggT ^{3'}	5'-AgT ^{3'}	5'-ggT ^{3'}	5'-ggC ^{3'}	5'-ACg ^{3'}		
	446	704	362				362			
	5'-CgA ^{3'}	5'-TgT ^{3'}	5'-ggT ^{3'}				5'-ggT ^{3'}			
256	256	463	302	527	256	559	259	463	3'-primer(s) ³	
5'-CCC ^{3'}	5'-CCC ^{3'}	5'-gCT ^{3'}	5'-ggC ^{3'}	5'-CCT ^{3'}	5'-CCC ^{3'}	5'-CTC ^{3'}	5'-CTT ^{3'}	5'-gCT ^{3'}		
	539	774	481	537			559			
	5'-TCA ^{3'}	5'-ggT ^{3'}	5'-gTA ^{3'}	5'-Agg ^{3'}			5'-CgT ^{3'}			
13	14	15	16	17	18	19	20	21	Well No.	
HLA-B allele ⁴										
13									*570101-570104, 570106	
13									*570105	
13									*570201, 570202	
13									*570301, 570302	
13									*5704	
13									*5705	
13									*5706	
13									*5707	
13									*5708	
13									*5709	
13	14								*5710	
13		15							*5711	
13			16						*5712	
13				17		19			*5713	
13									*5714	
13									*5715	
13	14	15	16	17	18	19	20	21	Well No.	

Lot No.: **66G**

Lot-specific information

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Length of spec.	150	100	220	100	220	95	105	90	170	210	165	90
PCR product						180		250			215	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*5716	1	2	3					8				
*5717	1		3	4								12
*5718	1	2	3									
*5719	1	2			5							
*5720	1	2	3									
*5721	1	2	3									
*5722	1	2	3						9			
*5723	1	2	3									
*5724	1	2									11	
*5725	1	2	3									
*5726	1	2	3									
*5727	1	2	3									
*5729	1	2	3									
*070201-0724, 0726-0747, 0749N, 0750, 0752-0799, 0806, 0820, 0853, 1316, 1331, 1405-140602, 1408, 1413, 15010101-150104, 150106-150116, 150301-1504, 1506-1512, 1514-1519, 1523, 1524, 1526N-1530, 1532-1535, 1537-1540, 1542, 1543, 1545-1547, 1549, 1550, 1553, 1554, 1556-1558, 1560, 1561, 1563-1574, 1576, 157801-1579N, 1581, 1582, 1587, 1590, 1592-1599, 9501-9504, 9508-9510, 9513, 9515-9520, 9522, 9525-9529, 9531-9535, 9537, 9538, 9540-9543, 9546-9550, 9552, 9553, 9556-9561, 9563, 9564, 9566-9569, 9571-9575, 9577, 9578, 9581N-9584, 1815, 1819, 1821, 1830, 270401-270403, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2725, 2740, 2754, 3543, 3544, 3558, 3567, 3579, 3586, 3599, 3707, 3810, 3918, 3935, 3936, 4005, 4015, 4016, 4023, 4026, 4032, 4051, 4095, 4098, 460101-4605, 4607N, 4608, 4610, 4612, 4614-4617, 4620, 4622, 4805, 4808, 4815, 490101-4909, 500101-5002, 5004-5009, 510101-5103, 5106, 5107, 5111N-5114, 5116-5118, 5121-512403, 5126-5130, 5132-5139, 5141N, 5143, 5145, 5148-5152, 5155, 5157-5172, 5174-5180, 5182-5189, 520101-5202, 5204-5209, 5211-5218, 5406, 550101-550106, 5503, 5505, 5509, 5511, 5515, 5517, 5521, 5524, 5525, 5528, 5529, 5531, 5533, 5536, 5538, 5603, 560501-5606, 5621, 5625, 5808, 7801-7803, 7805, 7806									9			
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **66G**

Lot-specific information

www.olerup-ssp.com

90	135	110	85	210	165	240	90	150	Length of spec. PCR product
13	14	15	16	17	18	19	20	21	Well No.
	195	145	160				240		
13	14								*5716
13			16						*5717
13									*5718
13									*5719
							20		*5720
13								21	*5721
13				17					*5722
13					18				*5723
13									*5724
13						19			*5725
13							20		*5726
13				17					*5727
13		15							*5729
									*070201-0724, 0726-0747, 0749N, 0750, 0752-0799, 0806, 0820, 0853, 1316, 1331, 1405-140602, 1408, 1413, 15010101-150104, 150106-150116, 150301-1504, 1506-1512, 1514-1519, 1523, 1524, 1526N-1530, 1532-1535, 1537-1540, 1542, 1543, 1545-1547, 1549, 1550, 1553, 1554, 1556-1558, 1560, 1561, 1563-1574, 1576, 157801-1579N, 1581, 1582, 1587, 1590, 1592-1599, 9501-9504, 9508-9510, 9513, 9515-9520, 9522, 9525-9529, 9531-9535, 9537, 9538, 9540-9543, 9546-9550, 9552, 9553, 9556-9561, 9563, 9564, 9566-9569, 9571-9575, 9577, 9578, 9581N-9584, 1815, 1819, 1821, 1830, 270401-270403, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2725, 2740, 2754, 3543, 3544, 3558, 3567, 3579, 3586, 3599, 3707, 3810, 3918, 3935, 3936, 4005, 4015, 4016, 4023, 4026, 4032, 4051, 4095, 4098, 460101-4605, 4607N, 4608, 4610, 4612, 4614-4617, 4620, 4622, 4805, 4808, 4815, 490101-4909, 500101-5002, 5004-5009, 510101-5103, 5106, 5107, 5111N-5114, 5116-5118, 5121-512403, 5126-5130, 5132-5139, 5141N, 5143, 5145, 5148-5152, 5155, 5157-5172, 5174-5180, 5182-5189, 520101-5202, 5204-5209, 5211-5218, 5406, 550101-550106, 5503, 5505, 5509, 5511, 5515, 5517, 5521, 5524, 5525, 5528, 5529, 5531, 5533, 5536, 5538, 5603, 560501-5606, 5621, 5625, 5808, 7801-7803, 7805, 7806
13	14	15	16	17	18	19	20	21	Well No.

Length of spec.	150	100	220	100	220	95	105	90	170	210	165	90
PCR product						180		250			215	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*0849, 130101-130103, 1306, 1307N, 1312, 1317, 132201-1323, 1325, 1326, 1328, 1329, 1336, 1520, 1588, 1822, 2719, 2730, 350101-350119, 350301-350303, 3507-350804, 3510, 3513, 3515-3517, 3519-352002, 3523-3530, 3533-3536, 3538-3542, 3545-3550, 3552, 3554-3557, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3580, 3582, 3584, 3585, 3590-3594, 3598, 44020101-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-4461N, 4463-446402, 4466-4475, 4478, 4480, 4481, 4483-4498, 480201, 480202, 5142, 530101-5305, 5307, 5309-5313, 5315-5318, 5320, 5321, 5609, 5611, 580101, 580102, 580104, 5804, 5805, 5809-5813, 5815, 5817N, 5821-5824, 8301	1											
*1313, 350201-350204, 350401-350403, 351201, 351202, 3581, 3583, 3595, 4462, 4477, 4482, 5319, 5612, 5828	1			4								
*1320, 1410, 150201-150204, 1513, 1521, 152501, 152502, 1536, 1544, 1562, 1577, 1580, 1585, 1589, 9506, 9512, 9521, 9539, 9544, 9554, 9565, 9570, 3511, 351401, 351402, 3521, 3596, 4476, 4479, 5306, 530801, 530802, 5819	1								9			
*1321, 350901, 350902, 3518, 3588, 3712, 4004, 4064, 4068, 4099, 4213, <i>Cw*1524</i>				4								
*1401-1404, 1407N, 1409, 1411, 1412, 1414-1418, 5806									9			
*1844, 5802, 5807, 5825, <i>Cw*0132</i> , <i>Cw*0620</i>												
*3506, 3559, 370101-370107, 3703N-3706, 3708, 3710, 3711, 3714-3721, 3820, 3942	1			w								
*3713	1			w					9			
*4028, 5104, 5146, 5156	1			4					9			
*4030, 4034			3	4								12
*4817, <i>Cw*0510</i>				w								
*5514	1	2	3						9		11	
*5814	1	2	3								11	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **66G**

Lot-specific information

www.olerup-ssp.com

90	135	110	85	210	165	240	90	150	Length of spec. PCR product
13	14	15	16	17	18	19	20	21	Well No.
	195	145	160				240		
									*0849, 130101-130103, 1306, 1307N, 1312, 1317, 132201-1323, 1325, 1326, 1328, 1329, 1336, 1520, 1588, 1822, 2719, 2730, 350101-350119, 350301-350303, 3507-350804, 3510, 3513, 3515- 3517, 3519-352002, 3523-3530, 3533-3536, 3538- 3542, 3545-3550, 3552, 3554-3557, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3580, 3582, 3584, 3585, 3590-3594, 3598, 44020101-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442- 4446, 4448-4452N, 4455-4461N, 4463-446402, 4466-4475, 4478, 4480, 4481, 4483-4498, 480201, 480202, 5142, 530101-5305, 5307, 5309-5313, 5315-5318, 5320, 5321, 5609, 5611, 580101, 580102, 580104, 5804, 5805, 5809-5813, 5815, 5817N, 5821-5824, 8301
									*1313, 350201-350204, 350401-350403, 351201, 351202, 3581, 3583, 3595, 4462, 4477, 4482, 5319, 5612, 5828
									*1320, 1410, 150201-150204, 1513, 1521, 152501, 152502, 1536, 1544, 1562, 1577, 1580, 1585, 1589, 9506, 9512, 9521, 9539, 9544, 9554, 9565, 9570, 3511, 351401, 351402, 3521, 3596, 4476, 4479, 5306, 530801, 530802, 5819
									*1321, 350901, 350902, 3518, 3588, 3712, 4004, 4064, 4068, 4099, 4213, <i>Cw*1524</i>
		15							*1401-1404, 1407N, 1409, 1411, 1412, 1414-1418, 5806
		15							*1844, 5802, 5807, 5825, <i>Cw*0132</i> , <i>Cw*0620</i>
									*3506, 3559, 370101-370107, 3703N-3706, 3708, 3710, 3711, 3714-3721, 3820, 3942 *3713
									*4028, 5104, 5146, 5156
						19			*4030, 4034
									*4817, <i>Cw*0510</i>
				17			20		*5514
									*5814
13	14	15	16						Well No.

Lot No.: **66G**

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*57 subtyping.

In addition, wells number 4, 11 and 13 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

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

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

⁴The sequence of the B*5728N allele is not yet available

⁵Primer mix 6: Specific PCR fragment of 95 bp in the B*5704 allele. Specific PCR fragment of 180 bp in the B*5706 allele.

Primer mix 8: Specific PCR fragment of 90 bp in the B*5716 allele. Specific PCR fragment of 250 bp in the B*5707 allele.

Primer mix 11: Specific PCR fragment of 165 bp in the B*5714 and B*5514 and 5814 alleles. Specific PCR fragment of 215 bp in the B*5709 and 5724 alleles.

Primer mix 14: Specific PCR fragment of 135 bp in the B*5717 allele. Specific PCR fragment of 195 bp in the B*5710 allele.

Primer mix 15: Specific PCR fragment of 110 bp in the B*5729 allele. Specific PCR fragment of 145 bp in the B*5711, 1401-1404, 1407N, 1409, 1411, 1412, 1414-1418, 1844, 5802, 5806, 5807, 5825, Cw*0132, Cw*0620 alleles.

Primer mix 16: Specific PCR fragment of 85 bp in the B*5712 allele. Specific PCR fragment of 160 bp in the B*5718 allele.

Primer mix 20: Specific PCR fragment of 90 bp in the B*5720 allele. Specific PCR fragment of 240 bp in the B*5726 and the B*5514 alleles.

'w', might be weakly amplified.

CELL LINE VALIDATION SHEET																			
HLA-B*57 SSP subtyping kit																			
			Prod. No.:	Well															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	IHWC cell line	B*		200967301	200967302	200967303	200967304	200967305	200967306	200967307	200967308	200967309	200967310	200967311	200967312	200967313	200967314	200967315	200967316
1	9001 SA	*0702		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
2	9280 LK707	*5201	*7301	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
3	9011 E4181324	*52011		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
4	9275 GU373	*1510	*5301	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
5	9009 KAS011	*3701		+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM	*3901	*5101	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
7	9020 QBL	*1801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025 DEU	*3501		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3	*5401		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT	*440301		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*5701		+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-
13	9004 JESTHOM	*2705		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*1501	*1520	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
15	9075 DKB	*4001		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*4002		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540	*0801	*5501	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
18	9257 32367	*1401	*5601	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
19	9038 BM16	*1801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*4001		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*1501		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
22	9056 KOSE	*3503		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*4002	*5602	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*1402		-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
26	9285 WT49	*5801		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*0705	*5101	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
28	9320 BEL5GB	*4402	*4403	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*440301		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*4201		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*1801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*4102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*4001		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*4402		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*1501	*3501	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
37	9065 HHKB	*0702		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
38	9099 LZL	*1501		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
39	9315 CML	*0801	*2705	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*1302	*4601	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
41	9055 H0301	*1402		-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
42	9066 TAB089	*4601		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
43	9076 T7526	*4601		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
44	9057 TEM	*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*4201	*5001	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
46	9013 SCHU	*0702		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
47	9045 TUBO	*5101		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
48	9303 TER-ND	*3501	*4403	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CELL LINE VALIDATION SHEET									
HLA-B*57 SSP subtyping kit									
					Well				
					17	18	19	20	21
				Prod. No.:	200967317	200967318	200967319	200967320	200967321
	IHWC cell line		B*						
1	9001 SA		*0702		-	-	-	-	-
2	9280 LK707		*5201	*7301	-	-	-	-	-
3	9011 E4181324		*52011		-	-	-	-	-
4	9275 GU373		*1510	*5301	-	-	-	-	-
5	9009 KAS011		*3701		-	-	-	-	-
6	9353 SM		*3901	*5101	-	-	-	-	-
7	9020 QBL		*1801		-	-	-	-	-
8	9025 DEU		*3501		-	-	-	-	-
9	9026 YAR		*3801		-	-	-	-	-
10	9107 LKT3		*5401		-	-	-	-	-
11	9051 PITOUT		*440301		-	-	-	-	-
12	9052 DBB		*5701		-	-	-	-	-
13	9004 JESTHOM		*2705		-	-	-	-	-
14	9071 OLGA		*1501	*1520	-	-	-	-	-
15	9075 DKB		*4001		-	-	-	-	-
16	9037 SWEIG007		*4002		-	-	-	-	-
17	9282 CTM3953540		*0801	*5501	-	-	-	-	-
18	9257 32367		*1401	*5601	-	-	-	-	-
19	9038 BM16		*1801		-	-	-	-	-
20	9059 SLE005		*4001		-	-	-	-	-
21	9064 AMALA		*1501		-	-	-	-	-
22	9056 KOSE		*3503		-	-	-	-	-
23	9124 IHL		*4002	*5602	-	-	-	-	-
24	9035 JBUSH		*3801		-	-	-	-	-
25	9049 IBW9		*1402		-	-	-	-	-
26	9285 WT49		*5801		-	-	-	-	-
27	9191 CH1007		*0705	*5101	-	-	-	-	-
28	9320 BEL5GB		*4402	*4403	-	-	-	-	-
29	9050 MOU		*440301		-	-	-	-	-
30	9021 RSH		*4201		-	-	-	-	-
31	9019 DUCAF		*1801		-	-	-	-	-
32	9297 HAG		*4102		-	-	-	-	-
33	9098 MT14B		*4001		-	-	-	-	-
34	9104 DHIF		*3801		-	-	-	-	-
35	9302 SSTO		*4402		-	-	-	-	-
36	9024 KT17		*1501	*3501	-	-	-	-	-
37	9065 HHKB		*0702		-	-	-	-	-
38	9099 LZL		*1501		-	-	-	-	-
39	9315 CML		*0801	*2705	-	-	-	-	-
40	9134 WHONP199		*1302	*4601	-	-	-	-	-
41	9055 H0301		*1402		-	-	-	-	-
42	9066 TAB089		*4601		-	-	-	-	-
43	9076 T7526		*4601		-	-	-	-	-
44	9057 TEM		*3801		-	-	-	-	-
45	9239 SHJO		*4201	*5001	-	-	-	-	-
46	9013 SCHU		*0702		-	-	-	-	-
47	9045 TUBO		*5101		-	-	-	-	-
48	9303 TER-ND		*3501	*4403	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-B*57 SSP

Product number: 101.567-12 – including *Taq* polymerase
Lot number: 66G
Expiry date: 2012-February-01
Number of tests: 12
Number of wells per test: 21

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2009-673-01	9	2009-673-09	17	2009-673-17
2	2009-673-02	10	2009-673-10	18	2009-673-18
3	2009-673-03	11	2009-673-11	19	2009-673-19
4	2009-673-04	12	2009-673-12	20	2009-673-20
5	2009-673-05	13	2009-673-13	21	2009-673-21
6	2009-673-06	14	2009-673-14		
7	2009-673-07	15	2009-673-15		
8	2009-673-08	16	2009-673-16		

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

No DNAs carrying the alleles to be amplified by primer solutions 6, 7, 8, 11, 14 and 16 to 21 were available. The specificities of the primers in primer solutions 6, 8, 11, 14, 16, 17, 19 and 20 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 7 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solution 18 and 21 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. An additional 3'primer in primer solution 15 was tested by separately adding one 5'-primer.

In primer solutions 2, 6, 16 17 and 20 one of the 3'-primers was not possible to test, and in primer solution 8, 14 and 15 one of the 5'-primers was not possible to test.

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

Date of approval: 2010-February-16

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP[®] HLA-B*57
Product number: 101.567-12
Lot number: 66G

Intended use: HLA-B*57 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 9001:2008 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-February-16

Olle Olerup
Managing Director

Lot No.: **66G**

Lot-specific information

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