

Olerup SSP[®] HLA-B*18

Product number:	101.519-12 – including <i>Taq</i> polymerase
Lot number:	47E
Expiry date:	2010-May-01
Number of tests:	12
Number of wells per test:	24
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. 47E.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP[®]* HLA-B*18 LOT

The HLA-B*18 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP[®]* HLA-B*18 lot was made (**Lot No. X79**).

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

Well	5'-primer	3'-primer	rationale
4	Added	Added	Primer pair added for the B*1828 allele.
9	Added	-	Primer added for the B*1827 allele.

PRODUCT DESCRIPTION

HLA-B*18 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*1801 to B*1829 alleles.

PLATE LAYOUT

Each test consists of 24 PCR reactions in a 24 well cut PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24

The 24 well cut PCR plate is marked with 'B*18 47E'.

Well No. 1 is marked with '1'.

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*18 SSP subtypings will be influenced by most B*07, most B*08, three B*13, the B*14, several B*15, most B*27, several B*35, the B*37, most B*38, most B*39, several B*40, the B*41, the B*42, most B*44, the B*45, two B*46, the B*47, two B*48, the B*49, the B*50, most B*51, most B*52, four B*53, most B*54, most B*55, several B*56, four B*57, three B*58, the B*59, the B*67, the B*7301, most B*78 and the B*8301 alleles when present on the other haplotype. In addition, the A*6604 allele might be weakly amplified by primer mix 13 and the Cw*1519 and Cw*1520 alleles will be amplified by primer mixes 18 and 23.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*18 alleles, i.e. **B*1801 to B*1829**, recognized by the HLA Nomenclature Committee in April 2008¹ will give rise to unique amplification patterns by the primers in the HLA-B*18 subtyping kit².

The HLA-B*18 kit cannot distinguish the B*180101 and B*180103 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2008-April-08, release 2.21.0, www.ebi.ac.uk/imgt/hla.

²The B*1829, B*3932 and B*3943 alleles will give rise to identical amplification patterns with the HLA-B*18 subtyping kit. The B*1829 allele can be distinguished from the B*3932 and B*3943 alleles by the HLA-B low resolution and/or HLA-B*39 subtyping kits.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 28 HLA-B*18 alleles can be combined in 406 homozygous and heterozygous combinations. 232 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products generated by primer mixes 10, 14 and 23 were not considered in these calculations.

++++-----	-----	-----+---	1802,1803 = 1802,1828
+++-----	-+-----	-----+---	1802,1809 = 1802,1823N
+++-----	-----	-----+---	1801,1802 = 1802,1829
++-++---	-----+--	-----+---	1803,1804 = 1804,1828
++-+-+--	-----	-----+---	1803,1805 = 1805,1828
++-+---+	-----	-----+---	1803,1807 = 1807,1828
++-+----+	--+-----	-----+--	1803,1821 = 1821,1828
++-+----+	--+-----	-----+---	1803,1810 = 1810,1828
++-+-----	+-----	-----+---	1803,1808 = 1803,1827 = 1808,1828 = 1827,1828
++-+-----	-+-----	-----+---	1803,1823N = 1809,1828 = 1823N,1828
++-+-----	--+-----	-----+---	1803,1811 = 1811,1828
++-+-----	-----+---	-----+---	1803,1812 = 1812,1828
++-+-----	-----+--	-----+---	1803,1819 = 1819,1828
++-+-----	-----+---	-----+---	1803,1813 = 1813,1828
++-+-----	-----+--	-----+---	1803,1814 = 1803,1825 = 1814,1828 = 1825,1828
++-+-----	-----+--	-+-----	1803,1815 = 1815,1828
++-+-----	-----+--	-----+---	1803,1817N = 1817N,1828
++-+-----	-----	+-----	1803,1818 = 1818,1828
++-+-----	-----	--+-----	1803,1806 = 1806,1828
++-+-----	-----	-----+---	1803,1820 = 1820,1828
++-+-----	-----	-----+--	1803,1822 = 1822,1828
++-+-----	-----	-----+--	1803,1824 = 1824,1828
++-+-----	-----	-----+--	1803,1826 = 1826,1828
++-+-----	-----	-----+---	1801,1803 = 1801,1828 = 1803,1828 = 1828,1828 = 1828,1829
++-+-----	+-----+--	-----+---	1804,1808 = 1804,1827
++-+-----	-+-----	-----+---	1804,1809 = 1804,1823N
++-+-----	-----+--	-----+---	1801,1804 = 1804,1804 = 1804,1814 = 1804,1825 = 1804,1829
++-+-----	+-----	-----+---	1805,1808 = 1805,1827
++-+-----	-+-----	-----+---	1805,1809 = 1805,1823N
++-+-----	-----+--	-----+---	1805,1814 = 1805,1825
++-+-----	-----	-----+---	1801,1805 = 1805,1805 = 1805,1829
++-+-----	+-----	-----+---	1807,1808 = 1807,1827
++-+-----	-+-----	-----+---	1807,1809 = 1807,1823N
++-+-----	-----+--	-----+---	1807,1814 = 1807,1825
++-+-----	-----	-----+---	1801,1807 = 1807,1807 = 1807,1829
++-+-----	-+-----	-----+--	1809,1821 = 1821,1823N
++-+-----	-+-----	-----+---	1809,1810 = 1810,1823N
++-+-----	--+-----	-----+--	1801,1821 = 1810,1824 = 1821,1824 = 1821,1829
++-+-----	--+-----	-----+---	1801,1810 = 1810,1829
++-+-----	++-----	-----+---	1808,1809 = 1808,1823N = 1809,1827 = 1823N,1827
++-+-----	+-----	-----+---	1808,1812 = 1812,1827

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++-----	+----+--	-----	1808,1819 = 1819,1827
++-----	+----+--	-----	1808,1813 = 1813,1827
++-----	+----+--	-----	1808,1814 = 1808,1825 = 1825,1827
++-----	+----+--	-+---+--	1808,1815 = 1815,1827
++-----	+----+--	-----	1808,1817N = 1817N,1827
++-----	+----+--	+---+---	1808,1818 = 1818,1827
++-----	+----+--	-+---+--	1806,1808 = 1806,1827
++-----	+----+--	---+---	1808,1820 = 1820,1827
++-----	+----+--	-----+--	1808,1824 = 1824,1827
++-----	+----+--	-----+--	1808,1826 = 1826,1827
++-----	+----+--	-----+--	1801,1808 = 1801,1827 = 1808,1808 = 1808,1827 = 1808,1829 = 1827,1829
++-----	-++-----	-----+--	1809,1811 = 1811,1823N
++-----	-++-----	-----+--	1809,1812 = 1812,1823N
++-----	-++-----	-----+--	1809,1819 = 1819,1823N
++-----	-++-----	-----+--	1809,1813 = 1813,1823N
++-----	-++-----	-----+--	1809,1814 = 1809,1825 = 1814,1823N = 1823N,1825
++-----	-+-----+--	-+---+--	1809,1815 = 1815,1823N
++-----	-+-----+--	-----+--	1809,1817N = 1817N,1823N
++-----	-+-----+--	+---+---	1809,1818 = 1818,1823N
++-----	-+-----+--	-+---+--	1806,1809 = 1806,1823N
++-----	-+-----+--	---+---	1809,1820 = 1820,1823N
++-----	-+-----+--	-----+--	1809,1822 = 1822,1823N
++-----	-+-----+--	-----+--	1809,1824 = 1823N,1824
++-----	-+-----+--	-----+--	1809,1826 = 1823N,1826
++-----	-+-----+--	-----+--	1801,1809 = 1801,1823N = 1809,1823N = 1823N,1823N = 1823N,1829
++-----	--+-----	-----+--	1801,1811 = 1811,1829
++-----	---+---+--	-----+--	1812,1814 = 1812,1825
++-----	---+---+--	-----+--	1801,1812 = 1812,1812 = 1812,1829
++-----	-----+++	-----+--	1814,1819 = 1819,1825
++-----	-----+++	-----+--	1813,1814 = 1813,1825
++-----	-----+++	-+---+--	1813,1815 = 1815,1819
++-----	-----+++	-----+--	1801,1819 = 1813,1819 = 1819,1819 = 1819,1829
++-----	-----+---	-----+--	1801,1813 = 1813,1813 = 1813,1829
++-----	-----+---	-+---+--	1814,1815 = 1815,1825
++-----	-----+---	-----+--	1814,1817N = 1817N,1825
++-----	-----+---	+---+---	1814,1818 = 1818,1825
++-----	-----+---	-+---+--	1806,1814 = 1806,1825
++-----	-----+---	---+---	1814,1820 = 1820,1825
++-----	-----+---	-----+--	1814,1824 = 1824,1825
++-----	-----+---	-----+--	1814,1826 = 1825,1826
++-----	-----+---	-----+--	1801,1814 = 1801,1825 = 1814,1825 = 1814,1829 = 1825,1825 = 1825,1829
++-----	-----+---	-+---+--	1801,1815 = 1815,1815 = 1815,1829
++-----	-----+---	-----+--	1801,1817N = 1817N,1817N = 1817N,1829
++-----	-----+---	+---+---	1801,1818 = 1818,1818 = 1818,1829
++-----	-----+---	-+---+--	1801,1806 = 1806,1806 = 1806,1829
++-----	-----+---	---+---	1801,1820 = 1820,1829
++-----	-----+---	-----+--	1801,1822 = 1822,1829
++-----	-----+---	-----+--	1801,1824 = 1824,1824 = 1824,1829

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++-----	-----	----+--+	1801,1826 = 1826,1826 = 1826,1829
++-----	-----	----+---	1801,1801 = 1801,1829
+-----+	--+-----	-----+-	1810,1821 = 1811,1821 = 1821,1821
+-----+	--+-----	-----	1810,1810 = 1810,1811
-++-----	-----	----+---	1803,1803 = 1803,1829
-+-----	-+-----	----+---	1809,1809 = 1809,1829

SPECIFICITY TABLE

HLA-B*18 SSP subtyping

Specificities and sizes of the PCR products of the 24 primer mixes used for HLA-B*18 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*18 alleles	Other amplified HLA Class I alleles ³
1	170 bp	800 bp	180101-1802, 1804-1808, 1810-1815, 1817N-1828	
2^{4,5}	260 bp	800 bp	180101-180103, 1803-1809, 1812, 1813, 1815, 1817N- 1820, 1823N- 1826, 1828, 1829	140601-140602, 1408, 1537, 1538, 3932, 3943, 4051, 5106, 5145
3	260 bp	800 bp	1802	2728
4^{5,6,7}	100 bp	800 bp	1803, 1828	
5⁷	135 bp	1070 bp	1804	
6⁷	130 bp	1070 bp	1805	
7	150 bp	1070 bp	1807	
8	190 bp	1070 bp	1810, 1821	070201-0704, 0707-0713, 0715, 0716, 071801- 071802, 0721-0723, 0725-0727, 0729, 0730, 0733, 0735-0739, 0741, 0742, 0744-0752, 0754- 0759, 0761-0764, 1540, 1547, 1549, 1552, 9514, 9517, 9524, 9538, 2734, 2741, 3515, 3533, 3566, 3586, 3707, 4003, 4018, 4020, 4024, 4031-4033, 4044, 4052, 4059, 4606, 4808
9	255 bp	1070 bp	1808, 1827	
10^{6,7,8}	85, 125 bp	800 bp	1809, 1823N	
11	630 bp	1070 bp	1810, 1811, 1821	350202, 352002
12⁷	140 bp	1070 bp	1812	
13⁷	85 bp	1070 bp	1813, 1819	1403, 1538, 5129,

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				A*6604 ^{weakly}
14 ⁹	170, 260 bp	1070 bp	1804, 1814, 1825	1405, 350202, 352002
15 ⁷	95 bp	1070 bp	1815, 1819	1401-1408, 1537, 1538, 3521, 4028, 4051, 510101-510109, 5103, 5104, 5106, 5107, 5111N- 5114, 5116-5118, 5121, 5122, 512401-512402, 5126-5130, 5132, 5133, 5135, 5137-5139, 5141N, 5143, 5145, 5146, 5148- 5152, 5155, 520101- 5202, 5204-5209, 5212, 5306, 560501-5606, 5621, 5808, 7801-7803, 7805
16	365 bp	1070 bp	1817N	
17	255 bp	1070 bp	1818	3704 ^{weakly}
18	150 bp	1070 bp	1815	1503, 1516, 1518, 1523, 1529, 1539, 1540, 1561, 1562, 1564, 1567, 1569, 1572, 1574, 1580, 1593, 1595, 1598, 9503, 9508, 9515, 9519, 9527, 9531, 9532, 9534, 9538, 3511, 3521, 3558, 4608, 4904, 5137, 5145, 5306, 5308, 5514, 5806, Cw*1519, Cw*1520
19 ⁷	105 bp	1070 bp	1806	
20	285 bp	1070 bp	1820	0714, 0717, 0728, 0814, 0828, 1310, 1401-1408, 1502, 1506, 1513, 15170101-151702, 1521, 1525, 1531, 1536, 1544, 1555, 1577, 1588, 1589, 9506, 9512, 9521, 9528, 9537, 9539, 9544, 2701- 270402, 270502-2706, 2708-2710, 2712-2719, 2721, 2723, 2725, 2726, 2728-2732, 2735-2740, 2742, 370101-3706, 3708, 3710, 3711, 3713, 3917, 4009, 4042, 4060, 44020101-4409, 4411-

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				4413, 4415, 4416, 4418-4430, 4432-4442, 4444, 4446-4450, 4452N-4460, 4501-4508, 47010101-4705, 4817, 4901-4903, 4905, 5001, 5002, 5004, 5142, 5307, 5704, 5705, 7301, 8301
21	215 bp	1070 bp	180101-1809, 1812-1815, 1817N-1819, 1822-1829	1315, 1537, 1538, 3521, 3524, 3581, 3932, 3943, 4028, 4051, 510101-510109, 5103, 5104, 5106-510902, 5111N-5114, 5116, 5119-5122, 512401-512402, 5126-5133, 5135, 5137-5139, 5141N, 5143-5153, 5155, 520101-5202, 5204-5210, 5212, 5302, 5306, 560501-5606, 5621, 5714, 5808, 5809, 7801-7803, 7805
22	270 bp	800 bp	1822	3521, 3524, 3581, 3704, 4028, 5104, 5146, 5302, 5306, 5714, 5809
23^{5,7,10}	100, 145 bp	800 bp	1821, 1824	0709, 0711, 1533, 9507, 9516, 9541, 2741, 3564, 4003, 4020, 4052, 4059, 4606, 5713, Cw*1519, Cw*1520
24	190 bp	1070 bp	1826	0734, 080101-0806, 0808N-0810, 0812, 0813, 0815-0820, 0822-0824, 0826, 0827, 0829-0836, 1306, 1542, 1583, 1586, 3560, 3587, 3712, 380101, 380201-3807, 3809-3817, 39010101-39010102L, 390103-390602, 3908-3914, 3916, 3918-3920, 3922-3924, 3926-3928, 3930, 3931, 3934-3937, 3939, 3941, 3942, 4039, 4051, 4101-410302, 4105-4108, 4201, 4202, 4204-4208, 5121, 5136, 5401, 5402, 5404, 5405N, 5407,

5408N, 5410-5413,
550101-5505, 5507,
5510-5513, 5515-5521,
5525, 5526, 5528-5530,
5610, 5612, 5623, 5901,
5902, 670101-6702

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*18 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*18 subtyping.

In addition, wells number 2, 3, 4, 10, 22 and 23 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

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

³Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B*18 alleles will be amplified by primer mixes 2, 3, 8, 11, 13 to 15, 17, 18, 20 to 24. In addition, the A*6604 allele might be weakly amplified by primer mix 13 and the Cw*1519 and Cw*1520 alleles will be amplified by primer mixes 18 and 23.

The B*1829, B*3932 and B*3943 alleles will give rise to identical amplification patterns with the HLA-B*18 subtyping kit. The B*1829 allele can be distinguished from the B*3932 and B*3943 alleles by the HLA-B low resolution and/or HLA-B*39 subtyping kits.

⁴The B*1401 to 1404 and 1407N to 1408 alleles might be faintly amplified by primer mix 2.

⁵Primer mixes 2, 4 and 23 have a tendency of giving rise to primer oligomer formation.

⁶Primer mixes 4 and 10 have a tendency of giving rise to non-specific amplifications.

⁷Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁸Primer mix 10: Specific PCR fragment of 85 bp in the B*1823N allele. Specific PCR fragment of 125 bp in the B*1809 allele.

⁹Primer mix 14: Specific PCR fragment of 170 bp in the B*1804, B*1825 and the B*350202 and 352002 alleles. Specific PCR fragment of 260 bp in the B*1814 and B*1405 alleles.

¹⁰Primer mix 23: Specific PCR fragment of 100 bp in the B*1821 and B*0709, 0711, 1533, 9507, 9516, 9541, 2741, 3564, 4003, 4020, 4052, 4059, 4606, 5713, Cw*1519 and Cw*1520 alleles.

Specific PCR fragment of 145 bp in the B*1824 allele.

INTERPRETATION TABLE

HLA-B*18 SSP subtyping

Amplification patterns of the B*1801 to B*1829 alleles

	Well ⁵											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	170	260	260	100	135	130	150	190	255	85	630	140
PCR product(s)										125		
Length of int.	800	800	800	800	1070	1070	1070	1070	1070	800	1070	1070
pos. control ¹												
5'-primer(s) ²	161	363	363	234	141	363	161	412	368	234	234	161
	5'-Cgg3'	5'-Agg3'	5'-AAT3'	5'-CAA3'	5'-ATT3'	5'-Agg3'	5'-Cgg3'	5'-ATg3'	5'-gTg3'	5'-CAA3'	5'-CAA3'	5'-Cgg3'
				523					368	610		
				5'-CCA3'					5'-gTT3'	5'-TgT3'		
3'-primer(s) ³	292	583	583	292	234	453	272	559	583	317	583	259
	5'-gTA3'	5'-gTg3'	5'-gTg3'	5'-gTC3'	5'-TCT3'	5'-TCC3'	5'-TgA3'	5'-CTC3'	5'-gTg3'	5'-ggA3'	5'-gTA3'	5'-CTC3'
	292			583						3 rd I		
	5'-gTA3'			5'-gTg3'						5'-TAT3'		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-B allele ⁴												
*180101-180103	1	2										
*1802	1		3									
*1803		2		4								
*1804	1	2			5							
*1805	1	2				6						
*1806	1	2										
*1807	1	2					7					
*1808	1	2							9			
*1809		2								10		
*1810	1							8			11	
*1811	1										11	
*1812	1	2										12
*1813	1	2										
*1814	1											
*1815	1	2										
*1817N	1	2										
*1818	1	2										
*1819	1	2										
*1820	1	2										
*1821	1							8			11	
*1822	1											
*1823N	1	2								10		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE												
HLA-B*18 SSP subtyping												
Amplification patterns of the B*1801 to B*1829 alleles												
Well⁵												
13	14	15	16	17	18	19	20	21	22	23	24	
85	170	95	365	255	150	105	285	215	270	100	190	Length of spec.
	260									145		PCR product(s)
1070	1070	1070	1070	1070	1070	1070	1070	1070	800	800	1070	Length of int. pos. control ¹
539	106	527	41	368	419	234	409	409	355	130	412	5'-primer(s) ²
5'-gCg3'	5'-CCA3'	5'-TgA3'	5'-CTA3'	5'-gTC3'	5'-gTC3'	5'-CAA3'	5'-ggT3'	5'-ggC3'	5'-TCA3'	5'-AgT3'	5'-ATA3'	
539	363									419		
5'-gTg3'	5'-AgC3'									5'-gTC3'		
583	234	583	234	583	527	299	3 rd I	583	583	234	559	3'-primer(s) ³
5'-gTA3'	5'-TCT3'	5'-gTg3'	5'-TCT3'	5'-gTg3'	5'-CCT3'	5'-TCA3'	5'-TAT3'	5'-gTg3'	5'-gTg3'	5'-TCT3'	5'-CgT3'	
	583									477		
	5'-gTg3'									5'-gCg3'		
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												HLA-B allele ⁴
								21				*180101-180103
								21				*1802
								21				*1803
	14							21				*1804
								21				*1805
						19		21				*1806
								21				*1807
								21				*1808
								21				*1809
												*1810
												*1811
								21				*1812
13								21				*1813
	14							21				*1814
		15			18			21				*1815
			16					21				*1817N
				17				21				*1818
13		15						21				*1819
							20					*1820
										23		*1821
								21	22			*1822
								21				*1823N
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

Lot No.: **47E**

Lot-specific information

www.olerup.com

Length of spec.	170	260	260	100	135	130	150	190	255	85	630	140
PCR product(s)										125		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*1824	1	2										
*1825	1	2										
*1826	1	2										
*1827	1								9			
*1828	1	2		4								
*1829, 3932, 3943 ⁶		2										
*070201-0704, 0707, 0708, 0710, 0712, 0713, 0715, 0716, 071801-071802, 0721- 0723, 0725-0727, 0729, 0730, 0733, 0735-0739, 0741, 0742, 0744-0752, 0754-0759, 0761-0764, 1547, 1549, 1552, 9514, 9517. 9524, 2734, 3515, 3533, 3566, 3586, 3707, 4018, 4024, 4031-4033, 4044, 4808								8				
*0709, 0711, 2741, 4003, 4020, 4052, 4059, 4606, 5713								8				
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **47E**

Lot-specific information

www.olerup.com

85	170	95	365	255	150	105	285	215	270	100	190	Length of spec. PCR product(s)
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
	260									145		
								21		23		*1824
	14							21				*1825
								21			24	*1826
								21				*1827
								21				*1828
								21				*1829, 3932, 3943 ⁶
												*070201-0704, 0707, 0708, 0710, 0712, 0713, 0715, 0716, 071801-071802, 0721- 0723, 0725-0727, 0729, 0730, 0733, 0735-0739, 0741, 0742, 0744-0752, 0754-0759, 0761-0764, 1547, 1549, 1552, 9514, 9517. 9524, 2734, 3515, 3533, 3566, 3586, 3707, 4018, 4024, 4031-4033, 4044, 4808
										23		*0709, 0711, 2741, 4003, 4020, 4052, 4059, 4606, 5713
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

Lot No.: **47E**

Lot-specific information

www.olerup.com

Length of spec.	170	260	260	100	135	130	150	190	255	85	630	140
PCR product(s)										125		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*0714, 0717, 0728, 0814, 0828, 1310, 1502, 1506, 1513, 15170101-151702, 1521, 1525, 1531, 1536, 1544, 1555, 1577, 1588, 1589, 9506, 9512, 9521, 9528, 9537, 9539, 9544, 2701-270402, 270502-2706, 2708-2710, 2712-2719, 2721, 2723, 2725, 2726, 2729-2732, 2735-2740, 2742, 370101-3703N, 3705, 3706, 3708, 3710, 3711, 3713, 3917, 4009, 4042, 4060, 44020101-4409, 4411- 4413, 4415, 4416, 4418- 4430, 4432-4442, 4444, 4446-4450, 4452N-4458N, 4460, 4501-4508, 47010101- 4705, 4817, 4901-4903, 4905, 5001, 5002, 5004, 5142, 5307, 5704, 5705, 7301, 8301												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **47E**

Lot-specific information

www.olerup.com

85	170	95	365	255	150	105	285	215	270	100	190	Length of spec. PCR product(s)
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
	260									145		
							20					*0714, 0717, 0728, 0814, 0828, 1310, 1502, 1506, 1513, 15170101-151702, 1521, 1525, 1531, 1536, 1544, 1555, 1577, 1588, 1589, 9506, 9512, 9521, 9528, 9537, 9539, 9544, 2701-270402, 270502-2706, 2708-2710, 2712-2719, 2721, 2723, 2725, 2726, 2729-2732, 2735-2740, 2742, 370101-3703N, 3705, 3706, 3708, 3710, 3711, 3713, 3917, 4009, 4042, 4060, 44020101-4409, 4411, 4413, 4415, 4416, 4418- 4430, 4432-4442, 4444, 4446-4450, 4452N-4458N, 4460, 4501-4508, 47010101- 4705, 4817, 4901-4903, 4905, 5001, 5002, 5004, 5142, 5307, 5704, 5705, 7301, 8301
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

Lot No.: **47E**

Lot-specific information

www.olerup.com

Length of spec.	170	260	260	100	135	130	150	190	255	85	630	140
PCR product(s)										125		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*0734, 080101-0806, 0808N-0813, 0815-0820, 0822-0824, 0826, 0827, 0829-0836, 1306, 1542, 1583, 1586, 3560, 3587, 3712, 380101, 380201-3807, 3809-3817, 39010101-39010102L, 390103-390602, 3908-3914, 3916, 3918-3920, 3922-3928, 3930, 3931, 3934-3937, 3939, 3941, 3942, 4039, 4101-410302, 4105-4108, 4201, 4202, 4204-4208, 5136, 5401, 5402, 5404, 5405N, 5407, 5408N, 5410-5413, 550101-5505, 5507, 5510-5513, 5515-5521, 5525, 5526, 5528-5530, 5610, 5612, 5623, 5901, 5902, 670101-6702												
*1315, 5108-510902, 5119, 5120, 5131, 5144N, 5153, 5210												
*1401-140202, 1404, 1407N												
*1403												
*1405												
*140601-140602, 1408		2										
*1503, 1516, 1518, 1523, 1529, 1539, 1561, 1562, 1564, 1567, 1569, 1572, 1574, 1580, 1593, 1595, 1598, 9503, 9508, 9515, 9519, 9527, 9531, 9532, 9534, 3511, 3558, 4608, 4904, 5308, 5514, 5806												
*1533, 9507, 9516, 9541, 3564												
*1537, 5106		2										
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **47E**

Lot-specific information

www.olerup.com

85	170	95	365	255	150	105	285	215	270	100	190	Length of spec.	
	260									145		PCR product(s)	
13	14	15	16	17	18	19	20	21	22	23	24	Well No.	
												*0734, 080101-0806, 0808N-0813, 0815-0820, 0822-0824, 0826, 0827, 0829-0836, 1306, 1542, 1583, 1586, 3560, 3587, 3712, 380101, 380201-3807, 3809-3817, 39010101-39010102L, 390103-390602, 3908-3914, 3916, 3918-3920, 3922-3928, 3930, 3931, 3934-3937, 3939, 3941, 3942, 4039, 4101-410302, 4105-4108, 4201, 4202, 4204-4208, 5136, 5401, 5402, 5404, 5405N, 5407, 5408N, 5410-5413, 550101-5505, 5507, 5510-5513, 5515-5521, 5525, 5526, 5528-5530, 5610, 5612, 5623, 5901, 5902, 670101-6702	
								24					
								21				*1315, 5108-510902, 5119, 5120, 5131, 5144N, 5153, 5210	
												*1401-140202, 1404, 1407N	
13		15						20					*1403
	14	15						20					*1405
		15						20					*140601-140602, 1408
				18								*1503, 1516, 1518, 1523, 1529, 1539, 1561, 1562, 1564, 1567, 1569, 1572, 1574, 1580, 1593, 1595, 1598, 9503, 9508, 9515, 9519, 9527, 9531, 9532, 9534, 3511, 3558, 4608, 4904, 5308, 5514, 5806	
												23	*1533, 9507, 9516, 9541, 3564
								21				*1537, 5106	
13	14	15	16	17	18	19	20	21	22	23	24	Well No.	

Length of spec.	170	260	260	100	135	130	150	190	255	85	630	140
PCR product(s)										125		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*1538		2										
*1540, 9538								8				
*2728			3									
*350202, 352002											11	
*3521, 5306												
*3524, 3581, 5302, 5714, 5809												
*3704												
*4028, 5104, 5146												
*4051		2										
*4459						6						
*510101-510109, 5103, 5107, 5111N-5114, 5116, 5122, 512401-512402, 5126- 5128, 5130, 5132, 5133, 5135, 5138, 5139, 5141N, 5143, 5148-5152, 5155, 520101-5202, 5204-5209, 5212, 560501-5606, 5621, 5808, 7801-7803, 7805												
*5117, 5118												
*5121												
*5129												
*5137												
*5145		2										
HLA-B allele ⁴												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
A*6604												
Cw*1519, Cw*1520												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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*18 subtyping.

In addition, wells number 2, 3, 4, 10, 22 and 23 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 1st, 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

³The nucleotide position, in 2nd or 3rd exon 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.

85	170	95	365	255	150	105	285	215	270	100	190	Length of spec. PCR product(s)
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
	260									145		
13	14	15	16	17	18	19	20	21	22	23	24	
13		15						21				*1538
				18								*1540, 9538
							20					*2728
14												*350202, 352002
		15			18			21	22			*3521, 5306
								21	22			*3524, 3581, 5302, 5714, 5809
				w			20		22			*3704
		15						21	22			*4028, 5104, 5146
		15						21			24	*4051
					20							*4459
		15						21				*510101-510109, 5103, 5107, 5111N-5114, 5116, 5122, 512401-512402, 5126 5128, 5130, 5132, 5133, 5135, 5138, 5139, 5141N, 5143, 5148-5152, 5155, 520101-5202, 5204-5209, 5212, 560501-5606, 5621, 5808, 7801-7803, 7805
		15										*5117, 5118
		15						21			24	*5121
13		15						21				*5129
		15			18			21				*5137
		15			18			21				*5145
												HLA-B allele ⁴
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
w												A*6604
				18				23				Cw*1519, Cw*1520
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

⁴The sequence of the B*1816 allele has been found to be identical to B*1814.

⁵Primer mix 10: Specific PCR fragment of 85 bp in the B*1823N allele. Specific PCR fragment of 125 bp in the B*1809 allele.

Primer mix 14: Specific PCR fragment of 170 bp in the B*1804, B*1825 and the B*350202 and 352002 alleles. Specific PCR fragment of 260 bp in the B*1814 and B*1405 alleles.

Primer mix 23: Specific PCR fragment of 100 bp in the B*1821 and B*0709, 0711, 1533, 9507, 9516, 9541, 2741, 3564, 4003, 4020, 4052, 4059, 4606, 5713, Cw*1519 and Cw*1520 alleles. Specific PCR fragment of 145 bp in the B*1824 allele.

⁶The B*1829, B*3932 and B*3943 alleles will give rise to identical amplification patterns with the HLA-B*18 subtyping kit. The B*1829 allele can be distinguished from the B*3932 and B*3943 alleles by the HLA-B low resolution and/or HLA-B*39 subtyping kits.

'w', might be weakly amplified.

CELL LINE VALIDATION SHEET																				
HLA-B*18 SSP subtyping kit																				
				Well																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				200733901	200733902	200733903	200845104	200733905	200733906	200733907	200733908	200733909	200733910	200733911	200733912	200733913	200733914	200733915	200733916	
	IHWC cell line		B*	Prod. No.:																
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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

CELL LINE VALIDATION SHEET												
HLA-B*18 SSP subtyping kit												
				Prod. No.:	Well							
					17	18	19	20	21	22	23	24
					200733917	200733918	200733919	200733920	200733921	200733922	200733923	200733924
	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	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	-	-	-	+	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-B*18 SSP

Product number: 101.519-12 – including *Taq* polymerase
Lot number: 47E
Expiry date: 2010-May-01
Number of tests: 12
Number of wells per test: 24

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2007-339-01	9	2008-451-09	17	2007-339-17
2	2007-339-02	10	2007-339-10	18	2007-339-18
3	2007-339-03	11	2007-339-11	19	2007-339-19
4	2008-451-04	12	2007-339-12	20	2007-339-20
5	2007-339-05	13	2007-339-13	21	2007-339-21
6	2007-339-06	14	2007-339-14	22	2007-339-22
7	2007-339-07	15	2007-339-15	23	2007-339-23
8	2007-339-08	16	2007-339-16	24	2007-339-24

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 4 to 7, 9 to 14, 16 to 19 and 23 were available. The specificities of the primers in primer solutions 4, 5, 7, 9, 10, 11, 12, 13, 14, 17, 18, 19 and 23 were tested by separately adding one additional 5'-primer, respectively one or two additional 3'-primer. In primer solution 6 only the 5'-primer was possible to test, the 3'-primer was not possible to test. In primer solution 16 only the 3'-primer was possible to test, the 5'-primer was not possible to test.

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

Date of approval: 2009-May-26

Approved by:

Quality Control, Supervisor

Lot No.: **47E**

Lot-specific information

www.olerup.com

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*18
Product number: 101.519-12
Lot number: 47E

Intended use: HLA-B*18 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 9001:2000 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, 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-26

Olle Olerup
Managing Director

Lot No.: **47E**

Lot-specific information

www.olerup.com

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