

Olerup SSP[®] HLA-B*08

Product number:	101.513-24/04 – including <i>Taq</i> pol. 101.513-24u/04u - without <i>Taq</i> pol.
Lot number:	24E
Expiry date:	2010-February-01
Number of tests:	24 tests – Product No. 101.513-24 4 tests – Product No. 101.513-04
Number of wells per test:	32
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. 24E.

**CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®]
HLA-B*08 Lot**

The HLA-B*08 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP*[®] HLA-B*08 lot was made (Lot No. X33).

Four wells have been added to the HLA-B*08 kit,
wells **29 to 32**.

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

Well	5'-primer	3'-primer	rationale
8	Modified	-	Increased specificity of specific primer pair.
29	New	New	New primer pair for the B*0832 allele.
30	New	New	New primer pair for the B*0833 allele.
31	New	New	New primer pair for the B*0834 allele.
32	New	New	New primer pair for the B*0835 allele.

PRODUCT DESCRIPTION

HLA-B*08 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*0801 to B*0835 alleles.

PLATE LAYOUT

Each test consists of 32 PCR reactions in a 32 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
25	26	27	28	29	30	31	32

The 32 well cut PCR plate is marked with 'B*08'.

Well No. 1 is marked with the Lot No. '24E'.

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

Please note: When removing each 32 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

The interpretation of HLA-B*08 SSP subtypings will be influenced by the several, B*07 alleles, the B*1318 allele, the B*1405 allele, eleven B*15 alleles, two B*18 alleles, seven B*27 alleles, most B*35 alleles, the B*3709 allele, two B*38 alleles, several B*39 alleles, most B*40 alleles, the B*41 alleles, the B*42 alleles, six B*44 alleles, the B*4606 allele, most B*48 alleles, most B*51 alleles, the B*5206 alleles, most B*53 alleles, the B*5410 allele, five B*55 alleles, two B*57 allele, the B*59 alleles, the B*6702 allele, the B*7301 allele, most B*78 alleles and the B*81 alleles when present on the other haplotype. In addition, the Cw*0305, Cw*0325., Cw*0327 and Cw*0808 alleles will be amplified by the primer mix 15. The Cw*0738 allele will be amplified by primer mixes 14 and 16.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*08 alleles, i.e. **B*0801 to B*0835**, recognized by the HLA Nomenclature Committee in January 2008¹ will give rise to unique amplification patterns by the primers in the HLA-B*08 subtyping kit.

The HLA-B*08 subtyping primer set can not distinguish the B*080101 to B*080105 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2008-January-11, release 2.20.0, www.ebi.ac.uk/imgt/hla.

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RESOLUTION IN HOMO- AND HETEROZYGOTES

The 35 HLA-B*08 alleles can be combined in 630 homozygous and heterozygous combinations. 228 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products generated by primer mixes 20 and 23 were not considered in these calculations.

+++++--	-----	-----	-----	0802,0804 = 0802,0824
+++++---	--+-----	-----	-----	0802,0809 = 0802,0827
+++--+--	-----	-----	-----	0803,0804 = 0803,0824 =
				0804,0823 = 0823,0824
+++--++	-----	-----	-----	0803,0805 = 0805,0823
+++--++	-----	-----	-----	0803,0806 = 0806,0823
+++--++	-----	-----	-----	0803,0820 = 0820,0823
+++--+	+-----	-----	-----	0803,0807 = 0807,0823
+++--+	-+-----	-----	-----	0803,0808N = 0808N,0823
+++--+	--+-----	-----	-----	0803,0809 = 0803,0827 =
				0809,0823 = 0823,0827
+++--+	---+-----	-----	-----	0803,0810 = 0810,0823
+++--+	-----+---	-----	-----	0803,0811 = 0811,0823
+++--+	-----+--	--+-----	-----	0803,0816 = 0816,0823
+++--+	-----+--	-----	-----	0803,0812 = 0812,0823
+++--+	-----++	-----+--	-----	0803,0825 = 0823,0825
+++--+	-----++	-----	-----	0803,0813 = 0813,0823
+++--+	-----	+-----	-----	0803,0814 = 0814,0823
+++--+	-----	---+-----	-----	0803,0822 = 0822,0823
+++--+	-----	-----+--	-----	0803,0818 = 0818,0823
+++--+	-----	-----+--	-----	0803,0819N = 0819N,0823
+++--+	-----	-----+--	-----	0803,0821 = 0821,0823
+++--+	-----	-----	+-----+	0803,0835 = 0823,0835
+++--+	-----	-----	+-----	0803,0828 = 0823,0828
+++--+	-----	-----	-+-----	0803,0829 = 0823,0829
+++--+	-----	-----	--+-----	0803,0830N = 0823,0830N
+++--+	-----	-----	---+-----	0803,0831 = 0823,0831
+++--+	-----	-----	-----+--	0803,0832 = 0823,0832
+++--+	-----	-----	-----+--	0803,0833 = 0823,0833
+++--+	-----	-----	-----+--	0803,0834 = 0823,0834
+++--+	-----	-----	-----	0801,0803 = 0801,0823 =
				0803,0823 = 0823,0823
+++--+	-----	-----	-----	0804,0805 = 0805,0824
+++--+	-----	---+---	-----	0806,0817 = 0817,0820
+++--+	-----	-----+--	-----	0804,0806 = 0806,0824
+++--+	-----	-----	-----	0804,0820 = 0820,0824
+++--+	+-----	-----	-----	0804,0807 = 0807,0824
+++--+	-+-----	-----	-----	0804,0808N = 0808N,0824
+++--+	--+-----	---+---	-----	0809,0817 = 0817,0827
+++--+	--+-----	-----	-----	0804,0809 = 0804,0827 =
				0809,0824 = 0824,0827
+++--+	---+-----	-----	-----	0804,0810 = 0810,0824
+++--+	-----+---	-----	-----	0804,0811 = 0811,0824
+++--+	-----+--	--+-----	-----	0804,0816 = 0816,0824
+++--+	-----+--	-----	-----	0804,0812 = 0812,0824
+++--+	-----++	---+---	-----	0813,0817 = 0817,0825
+++--+	-----++	-----+--	-----	0804,0825 = 0824,0825

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+++--+-	-----++	-----	-----	0804,0813 = 0813,0824
+++--+-	-----	+-----	-----	0804,0814 = 0814,0824
+++--+-	-----	-+-----	-----	0804,0815 = 0815,0824
+++--+-	-----	---+---+	-----	0801,0817 = 0817,0821 =
				0817,0822 = 0817,0824
+++--+-	-----	---+-----	-----	0804,0822 = 0822,0824
+++--+-	-----	---+-----	-----	0804,0818 = 0818,0824
+++--+-	-----	---+-----	-----	0804,0819N = 0819N,0824
+++--+-	-----	---+-----	-----	0804,0821 = 0821,0824
+++--+-	-----	-----	+-----+	0804,0835 = 0824,0835
+++--+-	-----	-----	+-----	0804,0828 = 0824,0828
+++--+-	-----	-----	+-----	0804,0829 = 0824,0829
+++--+-	-----	-----	---+-----	0804,0830N = 0824,0830N
+++--+-	-----	-----	---+-----	0804,0831 = 0824,0831
+++--+-	-----	-----	---+-----	0804,0832 = 0824,0832
+++--+-	-----	-----	---+-----	0804,0833 = 0824,0833
+++--+-	-----	-----	---+-----	0804,0834 = 0824,0834
+++--+-	-----	-----	-----	0801,0804 = 0801,0824 =
				0804,0824 = 0824,0824
+++--+-	---+-----	-----	-----	0805,0809 = 0805,0827
+++--+-	-----	-----	-----	0801,0805 = 0805,0805
+++--+-	-----	---+-----	-----	0801,0806 = 0806,0821 =
				0820,0821
+++-----	+---+-----	-----	-----	0807,0809 = 0807,0827
+++-----	+-----	-----	-----	0801,0807 = 0807,0807
+++-----	-+-----	-----	-----	0808N,0809 = 0808N,0827
+++-----	-+-----	-----	-----	0801,0808N = 0808N,0808N
+++-----	---+-----	-----	-----	0809,0810 = 0810,0827
+++-----	---+-----	-----	-----	0809,0811 = 0811,0827
+++-----	---+-----	+-----	-----	0809,0814 = 0814,0827
+++-----	---+-----	+-----	-----	0809,0815 = 0815,0827
+++-----	---+-----	---+-----	-----	0809,0822 = 0822,0827
+++-----	---+-----	---+-----	-----	0809,0818 = 0818,0827
+++-----	---+-----	---+-----	-----	0809,0819N = 0819N,0827
+++-----	---+-----	---+-----	-----	0809,0821 = 0821,0827
+++-----	---+-----	---+-----	-----	0809,0826 = 0826,0827
+++-----	---+-----	-----	+-----+	0809,0835 = 0827,0835
+++-----	---+-----	-----	+-----	0809,0828 = 0827,0828
+++-----	---+-----	-----	+-----	0809,0829 = 0827,0829
+++-----	---+-----	-----	---+-----	0809,0830N = 0827,0830N
+++-----	---+-----	-----	---+-----	0809,0831 = 0827,0831
+++-----	---+-----	-----	---+-----	0809,0832 = 0827,0832
+++-----	---+-----	-----	---+-----	0809,0833 = 0827,0833
+++-----	---+-----	-----	---+-----	0809,0834 = 0827,0834
+++-----	---+-----	-----	-----	0801,0809 = 0801,0827 =
				0809,0827 = 0827,0827
+++-----	---+-----	-----	-----	0801,0810 = 0810,0810
+++-----	---+-----	-----	-----	0801,0811 = 0811,0811
+++-----	-----++	---+-----	-----	0801,0825 = 0813,0821 =
				0821,0825
+++-----	-----	+-----	-----	0801,0814 = 0814,0814
+++-----	-----	---+-----	-----	0801,0822 = 0822,0822
+++-----	-----	---+-----	-----	0801,0818 = 0818,0818

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+++-----	-----	-----+--	-----	0801,0819N = 0819N,0819N
+++-----	-----	-----+--	-----	0801,0821 = 0821,0821
+++-----	-----	-----	+-----+	0801,0835 = 0828,0835 =
				0835,0835
+++-----	-----	-----	+-----	0801,0828 = 0828,0828
+++-----	-----	-----	+-----	0801,0829 = 0829,0829
+++-----	-----	-----	---+-----	0801,0830N = 0830N,0830N
+++-----	-----	-----	---+-----	0801,0831 = 0831,0831
+++-----	-----	-----	---+-----	0801,0832 = 0832,0832
+++-----	-----	-----	---+-----	0801,0833 = 0833,0833
+++-----	-----	-----	---+-----	0801,0834 = 0834,0834
-++-----+	-----++	-----+-	-----	0806,0813 = 0806,0825 =
				0820,0825
-++-----+	-----	-----+-	-----	0806,0806 = 0806,0820
-++-----	-----+-	---+-----	-----	0812,0816 = 0816,0816
-++-----	-----++	-----+-	-----	0813,0825 = 0825,0825

SPECIFICITY TABLE**HLA-B*08 SSP subtyping**

Specificities and sizes of the PCR products of the 32 primer mixes used for HLA-B*08 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*08 alleles	Other amplified HLA Class I alleles ³
1	215 bp	800 bp	080101-0805, 0807, 0808N, 0810, 0811, 0814, 0815, 0817-0819N, 0821-0824, 0826-0835	0704, 0719, 0725, 3709, 4102, 4104, 4201, 4202, 420501-4209
2	215 bp	800 bp	080101-0803, 0805-0816, 0818-0825, 0827-0835	
3	245 bp	1070 bp	080101-080105, 0804-0814, 0816, 0818-0825, 0827-0835	0729, 3907 [?] , 3912
4⁴	255 bp	1070 bp	0802	4449
5	245 bp	1070 bp	0803, 0823	
6⁴	205 bp	1070 bp	0804, 0817, 0824	4449
7	215 bp	1070 bp	0805	
8⁴	170 bp	1070 bp	0806, 0820	070501-0706, 0732, 0740, 0753, 1318, 1530, 1558, 1573, 3518, 3544, 3908, 3911, 3918, 3933, 3936, 4015, 4016, 4023, 4805, 4819, 5105, 5129, 5410, 5509, 5521, 5702, 5712
9	165 bp	1070 bp	0807	0704, 0719, 0725, 1551, 3538, 3709, 4104, 4209, 4417, 4451
10	175 bp	800 bp	0808N	
11	295 bp	800 bp	0809, 0827	4101, 4105, 4107, 4204, 5520
12	385 bp	1070 bp	0810	

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13⁵	90 bp	1070 bp	0811	
14	290 bp	1070 bp	0812, 0816	0707, 0712, 0714, 071801-071802, 9538, 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, 4072-4074, 4076, 4077, 4079, 410301-410302, 4108, 4431, 4606, 480301- 480302, 4817, Cw*0738
15	215 bp	1070 bp	0813, 0825	0726, 0734, 0743, 0748, 1405, 1555, 1814, 1822, 2707, 2711, 2724, 2732- 2734, 3505, 3516, 3517, 3522, 3530, 3531, 3551, 3558, 3572, 3903, 3914, 3924, 3929, 400201- 4003, 4005, 4008, 4009, 4013, 4018, 4019, 4024, 4027, 4029, 4031, 4035, 4037, 4039, 4040, 4045, 4050, 4056- 4058, 4071, 4078, 4080, 4454, 480101- 480102, 4804, 4806, 4807, 4809-4816, 4818, 5314, 5504, 8101-8103, Cw*0305, Cw*0325, Cw*0327, Cw*0808
16^{4,5}	110 bp	800 bp	0813, 0825	0726, 0734, 0743, 0748, 9538, 2736, 400101-400106, 4007, 4010, 4012, 401401- 401403, 4021, 4022N, 4025, 4030, 4031, 4033, 4034, 4036, 4038, 4042, 4043,

				4045-4049, 4052, 4054, 4055, 4059-4063, 4065-4067, 4069, 4072-4074, 4076, 4077, 4079, 4080, 4431, 4606, 480101-480102, 480301-480302, 4806, 4807, 4809-4812, 4814-4818, 8101-8103, Cw*0738
17⁵	95 bp	1070 bp	0814	0728, 2732, 4454
18	425 bp	800 bp	0815	0713, 0715, 3927, 4073, 6702, 7301
19	415 bp	800 bp	0816	0713, 0715, 6702
20^{5,6}	140, 205 bp	1070 bp	0817, 0822	4449
21	465 bp	1070 bp	0818	7301
22⁵	130 bp	800 bp	0819N	
23^{5,7}	90, 235 bp	800 bp	0806, 0817, 0821, 0825	0720, 0724, 1557, 9535, 3527, 3556, 4063, 5512
24	215 bp	1070 bp	0826	0708, 0732, 0738, 1508, 1529, 1556, 350101-3519, 3521-3525, 3527, 3529-3549, 3551-3562, 3564-3571, 3574, 3575, 3577, 3579-3581, 3583, 3806, 3807, 4406, 4806, 510101-5106, 5108-5121, 5123, 5124, 5126-5132, 5134-5146, 5148-5152, 520601-520602, 530101-5308, 5310-5314, 5901, 5902, 7801-780202, 7804
25	160 bp	1070 bp	0828, 0835	1551, 4209, 4417
26	385 bp	1070 bp	0829	
27⁵	105 bp	1070 bp	0830N	
28⁵	85 bp	1070 bp	0831	
29	150 bp	1070 bp	0832	
30	285 bp	1070 bp	0833	
31	375 bp	1070 bp	0834	
32	230 bp	1070 bp	0835	0709, 0711, 9538,

3566, 4038, 4052, 4059, 4209, 4606, 4814
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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*08 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*08 subtyping.

In addition, wells number 2, 10, 11, 16, 18, 19, 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 some non-HLA-B*08 alleles will be amplified by primer mixes 1, 3, 8, 9, 11, 14 to 19, 21, 23 and 24. In addition, the Cw*0305, Cw*0325., Cw*0327 and Cw*0808 alleles will be amplified by the primer mix 15. The Cw*0738 allele will be amplified by primer mixes 14 and 16.

⁴Primer mixes 4, 6, 8 and 16 may have a tendency of giving rise to nonspecific amplifications.

⁵Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁶Primer mix 20: Specific PCR fragment of 140 bp in the B*0822 allele. Specific PCR fragment of 205 bp in the B*0817 and B*4449 alleles.

⁷Primer mix 23: Specific PCR fragment of 90 bp in the B*0821, 0825 and B*0720, 0724 and 4063 alleles. Specific PCR fragment of 235 bp in the B*0806, 0817 and the B*1557, 9535, 3527, 3556 and 5512 alleles.

INTERPRETATION TABLE**HLA-B*08 SSP subtyping****Amplification patterns of the B*0801 to B*0835 alleles**

	Well ⁴															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length of spec.	215	215	245	255	245	205	215	170	165	175	295	385	90	290	215	110
PCR product																
Length of int.	800	800	1070	1070	1070	1070	1070	1070	1070	800	800	1070	1070	1070	1070	800
pos. control ¹																
5'-primer(s) ²	363	97	97	97	97	97	97	412	412	475	355	44	560	363	363	539
	5'-AgC ^{3'} 5'	-TCg ^{3'} 5'	-TCg ^{3'} 5'	-TCg ^{3'} 5'	-TCg ^{3'} 5'	-TCg ^{3'} 5'	-TCg ^{3'} 5'	-ATA ^{3'} 5'	-ATg ^{3'} 5'	-ACg ^{3'} 5'	-TCC ^{3'} 5'	-ggC ^{3'} 5'	-CgC ^{3'} 5'	-Agg ^{3'} 5'	-AgC ^{3'} 5'	-gCT ^{3'} 5'
											357					
											5'-Tgg ^{3'}					
3'-primer(s) ³	538	272	299	309	292	251	269	539	538	610	610	259	610	610	539	610
	5'-gTC ^{3'} 5'	-TgA ^{3'} 5'	-TCT ^{3'} 5'	-gTg ^{3'} 5'	-gTA ^{3'} 5'	-CCg ^{3'} 5'	-Agg ^{3'} 5'	-TCC ^{3'} 5'	-gTC ^{3'} 5'	-CTC ^{3'} 5'	-CTC ^{3'} 5'	-gTC ^{3'} 5'	-CTC ^{3'} 5'	-CTC ^{3'} 5'	-TCA ^{3'} 5'	-CTC ^{3'} 5'
					309	272										
					5'-ATC ^{3'} 5'	-Tgg ^{3'}										
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
HLA-B allele																
*080101-080105	1	2	3													
*0802	1	2		4												
*0803	1	2			5											
*0804	1		3		6											
*0805	1	2	3				7									
*0806		2	3					8								
*0807	1	2	3						9							
*0808N	1	2	3							10						
*0809		2	3								11					
*0810	1	2	3									12				
*0811	1	2	3										13			
*0812		2	3											14		
*0813		2	3												15	16
*0814	1	2	3													
*0815	1	2														
*0816		2	3											14		
*0817	1				6											
*0818	1	2	3													
*0819N	1	2	3													
*0820		2	3					8								
*0821	1	2	3													
*0822	1	2	3													
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

INTERPRETATION TABLE**HLA-B*08 SSP subtyping****Amplification patterns of the B*0801 to B*0835 alleles**

Well ⁴																
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
95	425	415	140	465	130	90	215	160	385	105	85	150	285	375	230	Length of spec.
			205			235										PCR product
1070	800	800	1070	1070	800	800	1070	1070	1070	1070	1070	1070	1070	1070	1070	Length of int.
																pos. control ¹
363	44	44	97	44	636	106	97	419	317	363	610	97	364	319	419	5'-primer(s) ²
5'-AgC ^{3'}	5'-ggC ^{3'}	5'-ggC ^{3'}	5'-TCg ^{3'}	5'-ggC ^{3'}	5'-CAC ^{3'}	5'-CCA ^{3'}	5'-TCT ^{3'}	5'-gTC ^{3'}	5'-gCA ^{3'}	5'-AgC ^{3'}	5'-TgA ^{3'}	5'-TCg ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	5'-gTC ^{3'}	
			363			560										
			5'-AgC ^{3'}			5'-CCT ^{3'}										
418	299	289	261	341	724	302	272	538	419	426	3 rd I	206	610	412	610	3'-primer(s) ³
5'-gTC ^{3'}	5'-TCA ^{3'}	5'-AgC ^{3'}	5'-gTC ^{3'}	5'-CgT ^{3'}	5'-CTA ^{3'}	5'-ggT ^{3'}	5'-TgA ^{3'}	5'-gTC ^{3'}	5'-CgT ^{3'}	5'-TCT ^{3'}	5'-TA ^{3'}	5'-CCg ^{3'}	5'-CTC ^{3'}	5'-gTT ^{3'}	5'-CTC ^{3'}	
			461			610										
			5'-gCg ^{3'}			5'-CTC ^{3'}										
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.
																HLA-B allele
																*080101-080105
																*0802
																*0803
																*0804
																*0805
						23										*0806
																*0807
																*0808N
																*0809
																*0810
																*0811
																*0812
17																*0813
	18															*0814
		19														*0815
																*0816
			20			23										*0817
				21												*0818
					22											*0819N
																*0820
						23										*0821
			20													*0822
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.

Length of spec.	215	215	245	255	245	205	215	170	165	175	295	385	90	290	215	110
PCR product																
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*0823	1	2	3		5											
*0824	1	2	3		6											
*0825		2	3												15	16
*0826	1															
*0827	1	2	3								11					
*0828	1	2	3													
*0829	1	2	3													
*0830N	1	2	3													
*0831	1	2	3													
*0832	1	2	3													
*0833	1	2	3													
*0834	1	2	3													
*0835	1	2	3													
*0704, 0719, 0725, 3709, 4104	1								9							
*070501-0706, 0740, 0753, 1318, 1530, 1558, 1573, 3908, 3911, 3918, 3933, 3936, 4015, 4016, 4805, 4819, 5410, 5509, 5521, 5702, 5712								8								
*0707, 0712, 0714, 071801-071802, 410301-410302, 4108													14			
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

101.513-24/04 – including *Taq* polymerase101.513-24u/04u – without *Taq* polymeraseLot No.: **24E**

Lot-specific information

www.olerup.com

95	425	415	140	465	130	90	215	160	385	105	85	150	285	375	230	Length of spec.
			205			235										PCR product
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.
																*0823
																*0824
						23										*0825
						24										*0826
																*0827
								25								*0828
									26							*0829
									27							*0830N
											28					*0831
												29				*0832
													30			*0833
														31		*0834
								25							32	*0835
																*0704, 0719, 0725, 3709, 4104
																*070501-0706, 0740, 0753, 1318, 1530, 1558, 1573, 3908, 3911, 3918, 3933, 3936, 4015, 4016, 4805, 4819, 5410, 5509, 5521, 5702, 5712
																*0707, 0712, 0714, 071801-071802, 410301-410302, 4108
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.

Length of spec.	215	215	245	255	245	205	215	170	165	175	295	385	90	290	215	110
PCR product																
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*0708, 0738, 1508, 1529, 1556, 350101- 350402, 3506-3515, 3519, 3521, 3523- 3525, 3529, 3532- 3537, 3539-3543, 3545-3549, 3552- 3555, 3557, 3559- 3562, 3564, 3565Q, 3567-3571, 3574, 3575, 3577, 3579- 3581, 3583, 3806, 3807, 4406, 510101- 5104, 5106, 5108- 5121, 5123, 5124, 5126-5128, 5130- 5132, 5134-5146, 5148-5152, 520601- 520602, 530101- 5308, 5310-5313, 5901, 5902, 7801- 780202, 7804																
*0709, 0711																
*0713, 0715, 6702																
*0720, 0724, 1557, 9535, 5512																
*0726, 0734, 0743, 0748, 4031, 4045, 4080, 480101- 480102, 4807, 4809- 4812, 4815, 4816, 4818, 8101-8103															15	16
*0728																
*0729, 3912			3													
*0732, 3518, 3544, 5105, 5129								8								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

101.513-24/04 – including *Taq* polymerase101.513-24u/04u – without *Taq* polymeraseLot No.: **24E**

Lot-specific information

www.olerup.com

95	425	415	140	465	130	90	215	160	385	105	85	150	285	375	230	Length of spec.
			205			235										PCR product
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.
							24									*0708, 0738, 1508, 1529, 1556, 350101-350402, 3506-3515, 3519, 3521, 3523-3525, 3529, 3532-3537, 3539-3543, 3545-3549, 3552-3555, 3557, 3559-3562, 3564, 3565Q, 3567-3571, 3574, 3575, 3577, 3579-3581, 3583, 3806, 3807, 4406, 510101-5104, 5106, 5108-5121, 5123, 5124, 5126-5128, 5130-5132, 5134-5146, 5148-5152, 520601-520602, 530101-5308, 5310-5313, 5901, 5902, 7801-780202, 7804
															32	*0709, 0711
	18	19														*0713, 0715, 6702
						23										*0720, 0724, 1557, 9535, 5512
																*0726, 0734, 0743, 0748, 4031, 4045, 4080, 480101-480102, 4807, 4809-4812, 4815, 4816, 4818, 8101-8103
17																*0728
																*0729, 3912
						24										*0732, 3518, 3544, 5105, 5129
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.

Length of spec.	215	215	245	255	245	205	215	170	165	175	295	385	90	290	215	110
PCR product																
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*1405, 1555, 1814, 1822, 2707, 2711, 2724, 2733, 2734, 3572, 3903, 3914, 3924, 3929, 400201- 4003, 4005, 4008, 4009, 4013, 4018, 4019, 4024, 4027, 4029, 4035, 4037, 4039, 4040, 4050, 4056-4058, 4071, 4078, 4804, 4813, 5504															15	
*1551									9							
*9538, 4038, 4052, 4059, 4606														14		16
*2732, 4454															15	
*2736, 4030, 4034																16
*3505, 3516, 3517, 3522, 3530, 3531, 3551, 3558, 5314															15	
*3527, 3556																
*3538									9							
*3566																
*3907			?													
*3927																
*400101-400106, 4007, 4010, 4012, 401401-401403, 4021, 4022N, 4025, 4033, 4036, 4042, 4043, 4046-4049, 4054, 4055, 4060- 4062, 4065-4067, 4069, 4072, 4074, 4076, 4077, 4079, 4431, 480301- 480302, 4817														14		16
*4023								8						14		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

101.513-24/04 – including *Taq* polymerase101.513-24u/04u – without *Taq* polymeraseLot No.: **24E**

Lot-specific information

www.olerup.com

95	425	415	140	465	130	90	215	160	385	105	85	150	285	375	230	Length of spec.
			205			235										PCR product
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.
																*1405, 1555, 1814, 1822, 2707, 2711, 2724, 2733, 2734, 3572, 3903, 3914, 3924, 3929, 400201- 4003, 4005, 4008, 4009, 4013, 4018, 4019, 4024, 4027, 4029, 4035, 4037, 4039, 4040, 4050, 4056-4058, 4071, 4078, 4804, 4813, 5504
								25								*1551
															32	*9538, 4038, 4052, 4059, 4606
17																*2732, 4454
																*2736, 4030, 4034
							24									*3505, 3516, 3517, 3522, 3530, 3531, 3551, 3558, 5314
						23	24									*3527, 3556
							24									*3538
							24								32	*3566
																*3907
	18															*3927
																*400101-400106, 4007, 4010, 4012, 401401-401403, 4021, 4022N, 4025, 4033, 4036, 4042, 4043, 4046-4049, 4054, 4055, 4060- 4062, 4065-4067, 4069, 4072, 4074, 4076, 4077, 4079, 4431, 480301- 480302, 4817
																*4023
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.

Length of spec.	215	215	245	255	245	205	215	170	165	175	295	385	90	290	215	110
PCR product																
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*4063														14		16
*4073														14		16
*4101, 4105, 4107, 4204, 5520											11					
*4102, 4201, 4202, 420501-4208	1															
*4209	1								9							
*4417									9							
*4449				4		6										
*4451									9							
*4806															15	16
*4814															15	16
*7301																
HLA-B allele																
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cw*0305, 0325, 0327, 0808															15	
Cw*0738														14		16
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

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*08 subtyping.

In addition, wells number 2, 10, 11, 16, 18, 19, 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, 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 2nd, 3rd or 4th exon or the 3rd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

95	425	415	140	465	130	90	215	160	385	105	85	150	285	375	230	Length of spec.
			205			235										PCR product
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.
						23										*4063
	18															*4073
																*4101, 4105, 4107, 4204, 5520
																*4102, 4201, 4202, 420501-4208
								25							32	*4209
								25								*4417
			20													*4449
																*4451
						24										*4806
															32	*4814
	18			21												*7301
																HLA-B allele
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.
																Cw*0305, 0325, 0327, 0808
																Cw*0738
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.

⁴Primer mix 20: Specific PCR fragment of 140 bp in the B*0822 allele. Specific PCR fragment of 205 bp in the B*0817 and B*4449 alleles.

Primer mix 23: Specific PCR fragment of 90 bp in the B*0821, 0825 and B*0720, 0724 and 4063 alleles. Specific PCR fragment of 235 bp in the B*0806, 0817 and the B*1557, 9535, 3527, 3556 and 5512 alleles.

101.513-24u/04u – without *Taq* polymerase

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CERTIFICATE OF ANALYSIS**Olerup SSP® HLA-B*08 SSP**

Product number: 101.513-24/04 – including *Taq* pol.
 101.513-24/04u – without *Taq* pol.
Lot number: 24E
Expiry date: 2010-February-01
Number of tests: 24 tests – Product No. 101.513-24
 4 tests – Product No. 101.513-04
Number of wells per test: 32

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2005-146-01	13	2002-146-13	25	2007-297-25
2	2002-146-02	14	2005-146-14	26	2007-297-26
3	2002-146-03	15	2005-146-15	27	2007-297-27
4	2002-146-04	16	2005-146-16	28	2007-297-28
5	2005-146-05	17	2005-146-17	29	2008-429-29
6	2002-146-06	18	2005-146-18	30	2008-429-30
7	2002-146-07	19	2007-297-19	31	2008-429-31
8	2008-429-08	20	2005-146-20	32	2008-429-32
9	2002-146-09	21	2005-146-21		
10	2002-146-10	22	2005-146-22		
11	2002-146-11	23	2005-146-23		
12	2005-146-12	24	2005-146-24		

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, 5, 6, 7, 9 10, 11, 12, 13, 17, 19, 20, 22, 23 and 25 to 32 were available. The specificities of the primers in primer solutions 4, 5, 6, 9, 11, 17, 19, 20, 23, 25 and 32 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. The specificity of the 5'-primers in primer solutions 7, 12, 22, 27 and 29 were tested by separately adding one additional 3'-primer, the 3'-primers in these primer solutions were not possible to test. In primer solutions 10, 13, 26, 28, 30 and 31 it was only possible to test the 3'-primers, the 5'-primers were not possible to test.

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

Date of approval: 2008-March-10

Approved by:

Quality Control, Supervisor

Lot No.: **24E**

Lot-specific information

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Declaration of Conformity

Product name: Olerup SSP® HLA-B*08
Product number: 101.513-24/04, 101.513-24u/04u
Lot number: 24E

Intended use: HLA-B*08 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, 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
2008-March-10

Olle Olerup
Managing Director

101.513-24/04 – including *Taq* polymerase101.513-24u/04u – without *Taq* polymeraseLot No.: **24E**

Lot-specific information

www.olerup.com**ADDRESSES:****Manufacturer:****Olerup SSP AB**, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.**Tel:** +46-8-717 88 27**Fax:** +46-8-717 88 18**E-mail:** info-ssp@olerup.com**Web page:** <http://www.olerup.com>**Distributed by:****Olerup GmbH**, Löwengasse 47 / 6, AT-1030 Vienna, Austria.**Tel:** +43-1-710 15 00**Fax:** +43-1-710 15 00 10**E-mail:** support-at@olerup.com**Web page:** <http://www.olerup.com>**Olerup Inc.**, 901 S. Bolmar St., Suite R, West Chester, PA 19382**Tel:** 1-877-OLERUP1**Fax:** 610-344-7989**E-mail:** info.us@olerup.com**Web page:** <http://www.olerup.com>For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.