Olerup SSP® HLA-B*13

Product number:	**101.515-12 – including *Taq* polymerase**
Lot number:	**96E**
Expiry date:	**2010-July-01**
Number of tests:	**12**
Number of wells per test:	**16**
Storage - pre-aliquoted primers:	**dark at -20°C**
- PCR Master Mix:	**-20°C**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 96E.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® HLA-B*13 LOT

The HLA-B*13 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup* SSP® HLA-B*13 lot was made **(Lot No. X63)**.

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	Added	Added	Primer pair added for the B*1318 allele.
10	Added	Added	Primer pair added for the B*1321 allele.
13	Added	Added	Primer pair added for the B*1319 allele.

PRODUCT DESCRIPTION

HLA-B*13 SSP subtyping

CONTENT
The primer set contains 5'- and 3'-primers for identifying the B*1301 to B*1321 alleles.

PLATE LAYOUT
Each test consists of 16 PCR reactions in a 16 well PCR plate.

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
9	**10**	**11**	**12**	**13**	**14**	**15**	**16**

The 16 well cut PCR plate is marked with 'B*13'.
Well No. 1 is marked with the Lot No. '96E'.
The PCR plates are heat-sealed with a PCR-compatible foil.
Please note: When removing each 16 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION
The interpretation of HLA-B*13 subtypings will be influenced by seven B*07, most B*08, most B*15, the B*18, several B*27, most B*35, the B*38, most B*39, the B*40, the B*41, the B*42, several B*44, the B*45, the B*46, the B*47, the B*48, the B*49, the B*50, several B*51, the B*5208, most B*53, the B*54, the B*55, the B*56, most B*57, most B*58, the B*59, the B*67, the B*7301 and the B*82 alleles when present on the other haplotype. In addition, the Cw*0206 allele will be amplified by primer mix 12 and the Cw*0334 allele will be amplified by primer mix 3.

UNIQUELY IDENTIFIED ALLELES
All the HLA-B*13 alleles, i.e. **B*1301 to B*1321**, recognized by the HLA Nomenclature Committee in April 2008[1] will give rise to unique amplification patterns by the primers in the HLA-B*13 subtyping kit.
The HLA-B*13 kit cannot distinguish the B*130201 to B*130204 alleles.

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

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 21 HLA-B*13 alleles can be combined in 210 homozygous and heterozygous combinations. Seventy-eight of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products generated by primer mixes 5, 8, 10, 13 and 16 were not considered in these calculations.

```
++++++-+ --------   1307N,1316 = 1318,1320
++++++-- -------+   1315,1320 = 1316,1317
++++++-- --------   1301,1316 = 1302,1320 = 1316,1320
+++++--+ -------+   1307N,1315 = 1317,1318
+++++--+ --------   1301,1318 = 1302,1307N = 1307N,1318
+++++--- -+---+--   1302,1321 = 1309,1313 = 1309,1321
+++++--- -------+   1301,1315 = 1302,1317 = 1315,1317
+++-+--+ --------   1303,1307N = 1307N,1320
+++-+--- -+---+--   1303,1321 = 1320,1321
+++-+--- -----+--   1303,1313 = 1313,1320
+++-+--- -------+   1303,1317 = 1317,1320
+++-+--- --------   1301,1303 = 1301,1320 = 1303,1320 =
                    1320,1320
++++---+ --------   1301,1307N = 1307N,1307N
++++---- -+---+--   1301,1321 = 1313,1321 = 1321,1321
++++---- -----+--   1301,1313 = 1313,1313
++++---- -------+   1301,1317 = 1317,1317
++-++--+ --------   1303,1318 = 1316,1318
++-++--- +-------   1303,1308Q = 1308Q,1316
++-++--- -+------   1303,1309 = 1309,1316
++-++--- ----+---   1303,1319 = 1312,1316 = 1316,1319
++-++--- ------+-   1303,1314 = 1314,1316
++-++--- -------+   1303,1315 = 1315,1316
++-++--- --------   1302,1303 = 1302,1316 = 1303,1316 =
                    1316,1316
++-+---+ ----+---   1312,1318 = 1318,1319
++-+---+ --------   1302,1318 = 1318,1318
++-+---- +---+---   1308Q,1312 = 1308Q,1319
++-+---- +-------   1302,1308Q = 1308Q,1308Q
++-+---- -+--+---   1309,1312 = 1309,1319
++-+---- ----+-+-   1312,1314 = 1314,1319
++-+---- ----+--+   1312,1315 = 1315,1319
++-+---- ----+---   1302,1312 = 1302,1319 = 1312,1319 =
                    1319,1319
++-+---- ------+-   1302,1314 = 1314,1314
++-+---- -------+   1302,1315 = 1315,1315
```

SPECIFICITY TABLE

HLA-B*13 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-B*13 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified HLA-B*13 alleles	Other amplified HLA Class I alleles[3]
1	140 bp	**800 bp**	1301-1304, 1306-1308Q, 1310-1321	1536, 1589, 4408, 4457, 4460
2[4,5]	95 bp	**800 bp**	1301-130204, 1307N-1311, 1313-1321	-
3	245 bp	1070 bp	1301, 1307N, 1313, 1317, 1320, 1321	0712, 0714, 071801-071802, 2719, 2730, 3515, 3533, 3588, 4004, 4030, 4034, 4059, 4064, 4068, 4416, 4421, 4817, 5713, **Cw*0334**
4	245 bp	1070 bp	130201-130204, 1308Q, 1309, 1314-1316, 1318, 1319	2714, 40060101-400602, 4044, 4053, 4070, 4075, 4083, 4086, 5110, 5116, 5131, 5134, 5208, 5509, 5522, 5524, 7301
5[7]	150, 180 bp	**800 bp**	1303, 1316, 1320	1542, 1573, 4071, 4410, 4415, 4418, 4501, 4504-4507, 4611, 4901-4903, 5001, 5002, 5004, 5115, 5403, 550101-550104, 5503, 5505, 5509, 5511, 5515, 5521, 5524, 5525, 5529, 5601, 5602, 5604, 5607, 5608, 5613, 5614, 5616, 5617, 5620, 8201, 8202
6[4]	105 bp	1070 bp	1304	1504, 1516, 1567, 1595, 9537, 3537, 390601-390602, 3933, 3934, 4086, 4502, 4904, 4905, 511301-511302, 5137, 5513, 5523, 5622, 7301
7[6]	190 bp	1070 bp	1306	0734, 080101-0806, 0808N-0810, 0812, 0813, 0815-0820, 0822-0824, 0826,

				0827, 0829-0836, 1542, 1583, 1586, 1826, 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
8[6,8]	185, 225 bp	1070 bp	1307N, 1318	1504, 9537, 3933, 5105, 5129, 5154, 5410, 5509, 5521
9	230 bp	1070 bp	1308	
10[4,9]	105, 210 bp	**800 bp**	1309, 1321	070501-0706, 0732, 0734, 0740, 0753, 080102, 0806, 0820, 1553, 2707, 2708, 2711, 2712, 2718, 2720, 2724, 2726, 2727, 2733, 2740, 2742, 350902, 3518, 3531, 3563, 3584, 3588, 400101-4016, 4018-4040, 4042-4088, 4101-4104, 4106-4108, 4409, 4431, 4446, 4501-4508, 4610, 4702, 480101-480102, 480301-4807, 4809-4811, 4813, 4815, 4816, 4818, 4819, 5001, 5002, 5004, 5110, 512402, 5411, 5504, 5508, 7301, 8101-8104N
11	275 bp	**800 bp**	1304, 1310	0709, 0711, 0717, 0828, 0835, 15010101-1508, 151101-1516, 1518-1521, 1523-1529, 1531-1536, 1538-1540, 1543, 1544, 1546, 1547, 1549-1557, 1560-1562, 1564-1572,

				1574-1576, 1578-1582, 1584, 1585, 1587-1589, 1591-1598, 9501-9529, 9531, 9532, 9534-9636, 9538-9546, 180101-1815, 1817N-1825, 1827-1829, 2741, 350101-350107, 3505, 3507-350803, 3510, 3511, 351401-3517, 3519-3521, 3523-3530, 3532, 3535, 3537, 3540N-3543, 3545-3554, 3557, 3558, 3561-3564, 3566-3569, 3571, 3572, 3576-3580, 3582, 3586, 3907, 3943, 4003, 4020, 4038, 4052, 4059, 4060, 4209, 4417, 4443, 460101-4610, 4612-4614, 4802, 4814, 4904, 4905, 5137, 5145, 530101-5303, 5305, 5306, 5308-5316, 5406, 5409, 5514, 5523, 5603, 5609, 5618, 570101-570103, 5706, 5708, 5710, 5711, 5713-5716, 580101-5802, 5804-5807, 5809-5817N
12[4]	90 bp	1070 bp	1311	1573, 3917, 4048, 5602, 5604, 5610, **Cw*0206**
13[4,10]	90,180 bp	**800 bp**	1312, 1319	
14[4]	130 bp	1070 bp	1313, 1321	
15	245 bp	1070 bp	1314	380201-3804, 3808, 3815
16[4,11]	90, 205 bp	1070 bp	1315, 1317	3546

[1]Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of HLA-B*13 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 inherit feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low

amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

[2]The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*13 subtyping.

In addition, wells number 2, 5, 10, 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.

[3]Due to the sharing of sequence motifs between HLA-B alleles some non-HLA-B*13 alleles will be amplified by primer mixes 1, 3 to 8 and 10 to 12, 15 and 16. In addition, the Cw*0206 allele will be amplified by primer mix 12 and the Cw*0334 allele will be amplified by primer mix 3.

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

[5]Primer mix 2 may give rise to a weak band of about 400 bp. This band should be disregarded when interpreting HLA-B*13 SSP subtypings.

[6]Primer mixes 7 and 8 has a weak tendency of nonspecific amplifications.

[7]Primer mix 5: Specific PCR fragment of 150 bp in the B*1316, 1320 and B*1542, 4611, 550101-550104, 5503, 5505, 5509, 5511, 5515, 5521, 5524, 5525 and 5529 alleles. Specific PCR fragment of 180 bp in the B*1303 and B*1573, 4071, 4410, 4415, 4418, 4501, 4504-4507, 5115, 5403, 5601, 5602, 5604, 5607, 5608, 5613, 5614, 5616, 5617, 5620, 8201 and 8202 alleles. Specific PCR fragments of 150 and 180 bp in the B*4901-4903, 5001, 5002 and 5004 alleles.

[8]Primer mix 8: Specific PCR fragment of 185 bp in the B*1307N allele. Specific PCR fragment of 225 bp in the B*1318 and the B*1504, 9537, 3933, 5105, 5129, 5154, 5410, 5509 and 5521 alleles.

[9]Primer mix 10: Specific PCR fragment of 105 bp in the B*1321 and the B*070501-0706, 0732, 0734, 0740, 0753, 080102, 0806, 0820, 2707, 2711, 2720, 2724, 2727, 2740, 2742, 350902, 3518, 3531, 4431, 480101-480102, 480301-4807, 4809-4811, 4813, 4815, 4816, 4818, 4819, 5110, 512402, 5411, 5504, 5508 and 8101-8104N alleles.

Specific PCR fragment of 210 bp in the B*1309 and the B*1553, 2708, 2712, 2718, 2726, 2733, 3563, 3584, 3588, 4101-4104, 4106-4108, 4409, 4446, 4501-4508, 4610, 4702, 5001, 5002, 5004 and 7301 alleles.

Specific PCR fragments of 105 and 210 bp in the B*400101-4016, 4018-4040, 4042-4088 alleles.

[10]Primer mix 13: Specific PCR fragment of 90 bp in the B*1319 allele. Specific PCR frgment of 180 bp in the B*1312 allele.

[11]Primer mix 16: Specific PCR fragment of 90 bp in the B*1317 and B*3546 alleles. Specific PCR fragment of 205 bp in the B*1315 allele.

INTERPRETATION TABLE

HLA-B*13 SSP subtyping

Amplification patterns of the B*1301 to *1321 alleles

	Well[5]							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Length of spec.	**140**	**95**	**245**	**245**	**150**	**105**	**190**	**185**
PCR product(s)					**180**			**225**
Length of int.	**800**	**800**	**1070**	**1070**	**800**	**1070**	**1070**	**1070**
pos. control[1]								
5'-primer(s)[2]	**209**	**506**	**355**	**357**	**420**	**357**	**412**	**106**
	5'-ggC3'	**5'-gCT3'**	**5'-TCA3'**	**5'-Tgg3'**	**5'-TTA3'**	**5'-Tgg3'**	**5'-ATA3'**	**5'-CCA3'**
								357
								5'-Tgg3'
3'-primer(s)[3]	**309**	**559**	**559**	**559**	**527**	**419**	**559**	**251**
	5'-gTg3'	**5'-CTC3'**	**5'-CTC3'**	**5'-CTC3'**	**5'-CCT3'**	**5'-Cgg3'**	**5'-CgT3'**	**5'-CCC3'**
					559	**419**		**539**
					5'-CAg3'	**5'-CgA3'**		**5'-TCC3'**
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
HLA-B allele[4]								
***1301**	**1**	**2**	**3**					
***130201-130204**	**1**	**2**		**4**				
***1303**	**1**				**5**			
***1304**	**1**					**6**		
***1306**	**1**						**7**	
***1307N**	**1**	**2**	**3**					**8**
***1308Q**	**1**	**2**		**4**				
***1309**		**2**		**4**				
***1310**	**1**	**2**						
***1311**	**1**	**2**						
***1312**	**1**							
***1313**	**1**	**2**	**3**					
***1314**	**1**	**2**		**4**				
***1315**	**1**	**2**		**4**				
***1316**	**1**	**2**		**4**	**5**			
***1317**	**1**	**2**	**3**					
***1318**	**1**	**2**		**4**				**8**
***1319**	**1**	**2**		**4**				
***1320**	**1**	**2**	**3**		**5**			
***1321**	**1**	**2**	**3**					
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**

INTERPRETATION TABLE

HLA-B*13 SSP subtyping

Amplification patterns of the B*1301 to *1321 alleles

Well[5]								
9	10	11	12	13	14	15	16	
230	105	275	90	90	130	245	90	Length of spec.
	210			180			205	PCR product(s)
1070	800	800	1070	800	1070	1070	1070	Length of int.
								pos. control[1]
357	142	419	369	268	419	103	144	5'-primer(s)[2]
5'-Tgg3'	5'-TCA3'	5'-gTC3'	5'-TAC3'	5'-Agg3'	5'-gTA3'	5'-CCT3'	5'-gCA3'	
	412			420			420	
	5'-ATA3'			5'-TTA3'			5'-TTA3'	
548	311	3rd I	420	317	506	309	193	3'-primer(s)[3]
5'-ggC3'	5'-ggT3'	5'-TAT3'	5'-gCT3'	5'-ggA3'	5'-TgA3'	5'-gTg3'	5'-CgT3'	
	477			559			420	
	5'-gCg3'			5'-CCC3'			5'-TTA3'	
9	10	11	12	13	14	15	16	Well No.
								HLA-B allele[4]
								*1301
								*130201-130204
								*1303
		11						*1304
								*1306
								*1307N
9								*1308Q
	10							*1309
		11						*1310
			12					*1311
				13				*1312
					14			*1313
						15		*1314
							16	*1315
								*1316
							16	*1317
								*1318
				13				*1319
								*1320
	10				14			*1321
9	10	11	12	13	14	15	16	Well No.

Length of spec.	**140**	**95**	**245**	**245**	**150**	**105**	**190**	**185**
PCR product(s)					**180**			**225**
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
***070501-0706, 0732, 0740, 0753, 2707, 2708, 2712, 2713, 2718, 2720, 2724, 2726, 2727, 2733, 2740, 2742, 350902, 3518, 3531, 3584, 400101-400203, 4005, 4007-4016, 4018, 4019, 4021-4029, 4031-4033, 4035-4037, 4040, 4042, 4043, 4045-4047, 4049, 4050, 4054-4058, 4061-4063, 4065-4067, 4069, 4072-4074, 4076-4082, 4084, 4085, 4087, 4088, 4104, 4409, 4431, 4446, 4503, 4508, 4702, 480101-480102, 480301-4811, 4813, 4815, 4816, 4818, 4819, 510109, 512402, 5508, 8101-8104N**								
***0709, 0711, 0717, 0828, 15010101-1503, 1505-1508, 151101-1515, 1518-1521, 1523-1529, 1531-1535, 1538-1540, 1543, 1544, 1546, 1547, 1549-1552, 1554-1557, 1560-1562, 1564-1566, 1568-1572, 1574-1576, 1578-1582, 1584, 1585, 1587, 1588, 1591-1594N, 1596-1598, 9501-9529, 9531, 9532, 9534-9536, 9538-9546, 180101-1815, 1817N-1825, 1827-1829, 2741, 350101-350107, 3505, 3507-350803, 3510, 3511, 351401-351402, 3516, 3517, 3519-3521, 3523-3530, 3532, 3535, 3540N-3543, 3545, 3547-3554, 3557, 3558, 3561, 3562, 3564, 3566-3569, 3571, 3572, 3576-3580, 3582, 3588, 3907, 3943, 4209, 4417, 4443, 460101-4609, 4612-4614, 4802, 4814, 5145, 530101-5303, 5305, 5306, 5308-5316, 5406, 5409, 5514, 5603, 5609, 5618, 570101-570103, 5706, 5708, 5710, 5711, 5714-5716, 5801-5802, 5804-5807, 5809-5817N**								
***0712, 0714, 071801-071802, 2719, 2730, 3533, 4416, 4421, 4817**			**3**					
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**

230	105	275	90	90	130	245	90	Length of spec.
	210			180			205	PCR product(s)
9	10	11	12	13	14	15	16	Well No.
	10							*070501-0706, 0732, 0740, 0753, 2707, 2708, 2712, 2713, 2718, 2720, 2724, 2726, 2727, 2733, 2740, 2742, 350902, 3518, 3531, 3584, 400101-400203, 4005, 4007-4016, 4018, 4019, 4021-4029, 4031-4033, 4035-4037, 4040, 4042, 4043, 4045-4047, 4049, 4050, 4054-4058, 4061-4063, 4065-4067, 4069, 4072-4074, 4076-4082, 4084, 4085, 4087, 4088, 4104, 4409, 4431, 4446, 4503, 4508, 4702, 480101-480102, 480301-4811, 4813, 4815, 4816, 4818, 4819, 510109, 512402, 5508, 8101-8104N
		11						*0709, 0711, 0717, 0828, 15010101-1503, 1505-1508, 151101-1515, 1518-1521, 1523-1529, 1531-1535, 1538-1540, 1543, 1544, 1546, 1547, 1549-1552, 1554-1557, 1560-1562, 1564-1566, 1568-1572, 1574-1576, 1578-1582, 1584, 1585, 1587, 1588, 1591-1594N, 1596-1598, 9501-9529, 9531, 9532, 9534-9536, 9538-9546, 180101-1815, 1817N-1825, 1827-1829, 2741, 350101-350107, 3505, 3507-350803, 3510, 3511, 351401-351402, 3516, 3517, 3519-3521, 3523-3530, 3532, 3535, 3540N-3543, 3545, 3547-3554, 3557, 3558, 3561, 3562, 3564, 3566-3569, 3571, 3572, 3576-3580, 3582, 3588, 3907, 3943, 4209, 4417, 4443, 460101-4609, 4612-4614, 4802, 4814, 5145, 530101-5303, 5305, 5306, 5308-5316, 5406, 5409, 5514, 5603, 5609, 5618, 570101-570103, 5706, 5708, 5710, 5711, 5714-5716, 5801-5802, 5804-5807, 5809-5817N
								*0712, 0714, 071801-071802, 2719, 2730, 3533, 4416, 4421, 4817
9	10	11	12	13	14	15	16	Well No.

Length of spec.	**140**	**95**	**245**	**245**	**150**	**105**	**190**	**185**
PCR product(s)					**180**			**225**
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
***0734, 080101, 080103-0805, 0808N-0810, 0812, 0813, 0815-0819N, 0822-0824, 0826, 0827, 0829-0834, 0836, 1583, 1586, 1826, 3560, 3587, 3712, 380101, 3805-3807, 3809-3814, 3816, 3817, 39010101-39010102L, 390103-3905, 3908-3914, 3916, 3918-3920, 3922-3924, 3926-3928, 3930, 3931, 3935-3937, 3939, 3941, 3942, 4105, 4201, 4202, 4204-4208, 5121, 5136, 5401, 5402, 5404, 5405N, 5407, 5408N, 5412, 5413, 550201-550202, 5507, 5510, 5512, 5516-5520, 5526, 5528, 5530, 5612, 5623, 5901, 5902, 670101-6702**							**7**	
***0734, 080102, 0806, 0820, 4039, 4051, 4101-410302, 4106-4108, 5411, 5504**							**7**	
***0835**							**7**	
***1504**						**6**		**8**
***1516, 1567, 1595, 3537, 4904, 4905, 5137, 5523**						**6**		
***1536, 1589**	**1**							
***1542, 550101-550104, 5503, 5505, 5511, 5515, 5525, 5529**					**5**		**7**	
***1553, 3563, 4003, 4020, 4038, 4052, 4060, 4610**								
***1573, 5602, 5604**					**5**			
***9537, 3933**						**6**		**8**
***2714, 5116, 5131, 5134, 5208, 5522**				**4**				
***3515, 5713**			**3**					
***3546**								
***3588, 4004, 4030, 4034, 4064, 4068**			**3**					
***380201-3804, 3815**							**7**	
***3808**								
***390601-390602, 3934, 5513**						**6**	**7**	
***3917**								
***40060101-400602, 4044, 4053, 4070, 4075, 4083, 5110**				**4**				
***4048**								
***4059**			**3**					
***4071, 4501, 4504-4507, 5001, 5002, 5004**					**5**			
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**

230	105	275	90	90	130	245	90	Length of spec.
	210			180			205	PCR product(s)
9	10	11	12	13	14	15	16	Well No.
								*0734, 080101, 080103-0805, 0808N-0810, 0812, 0813, 0815-0819N, 0822-0824, 0826, 0827, 0829-0834, 0836, 1583, 1586, 1826, 3560, 3587, 3712, 380101, 3805-3807, 3809-3814, 3816, 3817, 39010101-39010102L, 390103-3905, 3908 3914, 3916, 3918-3920, 3922-3924, 3926-3928, 3930, 3931, 3935-3937, 3939, 3941, 3942, 4105, 4201, 4202, 4204-4208, 5121, 5136, 5401, 5402, 5404, 5405N, 5407, 5408N, 5412, 5413, 550201-550202, 5507, 5510, 5512, 5516-5520, 5526, 5528, 5530, 5612, 5623, 5901, 5902, 670101-6702
	10							*0734, 080102, 0806, 0820, 4039, 4051, 4101-410302, 4106-4108, 5411, 5504
		11						*0835
		11						*1504
		11						*1516, 1567, 1595, 3537, 4904, 4905, 5137, 5523
		11						*1536, 1589
								*1542, 550101-550104, 5503, 5505, 5511, 5515, 5525, 5529
	10	11						*1553, 3563, 4003, 4020, 4038, 4052, 4060, 4610
			12					*1573, 5602, 5604
								*9537, 3933
								*2714, 5116, 5131, 5134, 5208, 5522
		11						*3515, 5713
		11					16	*3546
	10							*3588, 4004, 4030, 4034, 4064, 4068
						15		*380201-3804, 3815
						15		*3808
								*390601-390602, 3934, 5513
			12					*3917
	10							*40060101-400602, 4044, 4053, 4070, 4075, 4083, 5110
	10		12					*4048
	10	11						*4059
	10							*4071, 4501, 4504-4507, 5001, 5002, 5004
9	10	11	12	13	14	15	16	Well No.

Length of spec.	**140**	**95**	**245**	**245**	**150**	**105**	**190**	**185**
PCR product(s)					**180**			**225**
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
***4086, 7301**				**4**		**6**		
***4408, 4457, 4460**	**1**							
***4410, 4415, 4418, 4611, 4901-4903, 5115, 5403, 5601, 5607, 5608, 5613, 5614, 5616, 5617, 5620, 8201, 8202**					**5**			
***4502**						**6**		
***5105, 5129, 5154**								**8**
***511301-511302, 5622**						**6**		
***5410**							**7**	**8**
***5509**				**4**	**5**			**8**
***5521**					**5**		**7**	**8**
***5524**				**4**	**5**			
***5610**							**7**	
HLA-B allele[4]								
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Cw*0206								
Cw*0334			**3**					
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**

[1]The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.
Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*13 subtyping.
In addition, wells number 2, 5, 10, 11 and 13 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.
[2]The nucleotide position, in the 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.
[3]The nucleotide position, in 2nd or 3rd exon or the 3rd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.
[4]The sequence of the B*1305 allele has been shown to be identical to B*1304.

230	105	275	90	90	130	245	90	Length of spec.
	210			180			205	PCR product(s)
9	10	11	12	13	14	15	16	Well No.
	10							*4086, 7301
								*4408, 4457, 4460
								*4410, 4415, 4418, 4611, 4901-4903, 5115, 5403, 5601, 5607, 5608, 5613, 5614, 5616, 5617, 5620, 8201, 8202
	10							*4502
								*5105, 5129, 5154
								*511301-511302, 5622
								*5410
								*5509
								*5521
								*5524
			12					*5610
								HLA-B allele[4]
9	10	11	12	13	14	15	16	Well No.
			12					Cw*0206
								Cw*0334
9	10	11	12	13	14	15	16	Well No.

[5]Primer mix 5: Specific PCR fragment of 150 bp in the B*1316, 1320 and B*1542, 4611, 550101-550104, 5503, 5505, 5509, 5511, 5515, 5521, 5524, 5525 and 5529 alleles. Specific PCR fragment of 180 bp in the B*1303 and B*1573, 4071, 4410, 4415, 4418, 4501, 4504-4507, 5115, 5403, 5601, 5602, 5604, 5607, 5608, 5613, 5614, 5616, 5617, 5620, 8201 and 8202 alleles. Specific PCR fragments of 150 and 180 bp in the B*4901-4903, 5001, 5002 and 5004 alleles.
Primer mix 8: Specific PCR fragment of 185 bp in the B*1307N allele. Specific PCR fragment of 225 bp in the B*1318 and the B*1504, 9537, 3933, 5105, 5129, 5154, 5410, 5509 and 5521 alleles.
Primer mix 10: Specific PCR fragment of 105 bp in the B*1321 and the B*070501-0706, 0732, 0734, 0740, 0753, 080102, 0806, 0820, 2707, 2711, 2720, 2724, 2727, 2740, 2742, 350902, 3518, 3531, 4431, 480101-480102, 480301-4807, 4809-4811, 4813, 4815, 4816, 4818, 4819, 5110, 512402, 5411, 5504, 5508 and 8101-8104N alleles.
Specific PCR fragment of 210 bp in the B*1309 and the B*1553, 2708, 2712, 2718, 2726, 2733, 3563, 3584, 3588, 4101-4104, 4106-4108, 4409, 4446, 4501-4508, 4610, 4702, 5001, 5002, 5004 and 7301 alleles.
Specific PCR fragments of 105 and 210 bp in the B*400101-4016, 4018-4040, 4042-4088 alleles.
Primer mix 13: Specific PCR fragment of 90 bp in the B*1319 allele. Specific PCR frgment of 180 bp in the B*1312 allele.
Primer mix 16: Specific PCR fragment of 90 bp in the B*1317 and B*3546 alleles. Specific PCR fragment of 205 bp in the B*1315 allele.
'w', may be weakly amplified.

CELL LINE VALIDATION SHEET

HLA-B*13 SSP subtyping kit

					Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.:	200617501	200617502	200617503	200617504	200732405	200617506	200617507	2004980808	200617509	200849810	200617511	200617512	200849813	200617514	200617515	200723416
	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*13 SSP

Product number: **101.515-12 – including *Taq* polymerase**
Lot number: **96E**
Expiry date: **2010-July-01**
Number of tests: **12**
Number of wells per test: **16**

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2006-175-01	9	2006-175-09
2	2006-175-02	10	2008-498-10
3	2006-175-03	11	2006-175-11
4	2006-175-04	12	2006-175-12
5	2007-324-05	13	2008-498-13
6	2006-175-06	14	2006-175-14
7	2006-175-07	15	2006-175-15
8	2008-498-08	16	2007-234-16

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 3, 8, 9, 13, 14 and 16 were available. The specificities of the primers in primer solutions 3, 14 and 16 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 8, 9 and 13, it was only possible to test the 5'-primers, the 3'-primers were not possible to test.

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

Date of approval: 2009-May-25

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*13
Product number: 101.515-12
Lot number: 96E

Intended use: HLA-B*13 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-July-25

Olle Olerup
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