Olerup SSP® HLA-B*46

Product number:	**101.544-06u – without *Taq* polymerase**
Lot number:	**81G**
Expiry date:	**2012-February-01**
Number of tests:	**6**
Number of wells per test:	**16**
Storage - pre-aliquoted primers:	**dark at -20°C**
- PCR Master Mix:	**-20°C**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 81G.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® HLA-B*46 LOT

The HLA-B*46 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup* SSP® HLA-B*46 lot was made **(Lot No. 60F)**.

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

Well	5'-primer	3'-primer	rationale
6	Added	Added, exchanged	New primer pair for the B*4620 allele and exchanged 3'-primer.
8	-	Added	New primer for the B*4619 allele and exchanged positive control primer pair.
12	Added	Added	New primer pair for the B*4619 allele.
13	Added	Added	New primer pair for the B*4622 allele.
15	-	Added	New primer for the B*461302 allele.
16	Added	Added	New primer pair for the B*4622 allele.

PRODUCT DESCRIPTION

HLA-B*46 SSP typing

CONTENT
The primer set contains 5'- and 3'-primers for identifying the B*4601 to B*4622 alleles.

PLATE LAYOUT
Each HLA-B*46 test consists of 16 PCR reactions in a 16 well cut PCR plate.

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

The 16 well PCR plate is marked with 'HLA-B*46' in silver/gray ink.
Well No. 1 is marked with the Lot Number '81G'.
A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.
The PCR plates are heat-sealed with a PCR-compatible foil.
Please note: When removing each 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*46 SSP subtypings will be influenced by many other HLA-B alleles, in particular the HLA-B*15 alleles. In addition, the Cw*0738 allele will be amplified by primer mix 6, the Cw*0620 allele will be amplified by primer mix 9 and the Cw*0228 allele will be amplified by primer mix 11.

UNIQUELY IDENTIFIED ALLELES
All the HLA-B*46, i.e. **B*4601 to B*4622**, recognized by the HLA Nomenclature Committee in January 2010[1] will be amplified by the primers in the HLA-B*46 SSP kit.
The B*46 primer set cannot separate the B*460101 and B*460102 or the B*461301 and B*461302 alleles.

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

RESOLUTION IN HOMO- AND HETEROZYGOTES
The 22 HLA-B*46 alleles give rise to 23 different amplification patterns, that can be combined in 276 homozygous and heterozygous combinations. 159 of these genotypes do not give rise to unique amplification patterns. The different sizes of the specific PCR fragments generated by primer mixes 4, 6, 7 and 10, 12 and 13 were not considered in these calculations.

++-++-+- +-------	4604,4616 = 4614,4616
++-++--- +-------	4602,4604 = 4602,4614
++--+-+- ++------	4605,4616 = 4615N,4616
++--+-+- +-------	4601,4616 = 4602,4607N = 4602,4616 = 4607N,4616 = 4616,4616
++--+--- ++------	4602,4605 = 4602,4615N
++--+--- +-------	4601,4602 = 4602,4602
+-++--+ +---+-+-	4604,4618 = 4614,4618
+-++--+ +-------	4604,4621 = 4614,4621
+-+---- +-------	4603,4604 = 4603,4614
+-+-++-+ --------	4603,4606 = 4606,4621
+-+-+--+ ++--+-+-	4605,4618 = 4615N,4618
+-+-+--+ ++------	4605,4621 = 4615N,4621
+-+-+--+ +---+-++	4617,4618 = 4618,4622
+-+-+--+ +---+-+-	4601,4618 = 460103,4618
+-+-+--+ ---+----	4603,4619 = 4619,4621
+-+-+--+ ----+-+-	4603,4611 = 4603,4618 = 4608,4618 = 4611,4618 = 4611,4621 = 4613,4618 = 4618,4618 = 4618,4621
+-+-+--+ ------+-	4603,4613 = 4613,4621
+-+-+--+ --------	4603,4608 = 4603,4621 = 4608,4621 = 4621,4621
+-+-+--- ++------	4603,4605 = 4603,4615N
+--++-+ +-------	4604,4606 = 4606,4614
+--++-- +-------	4604,4620 = 4614,4620
+--++-+- +-------	4604,4607N = 4607N,4614
+--+--+ +--+----	4604,4619 = 4614,4619
+--+--+ +---+-+-	4604,4611 = 4611,4614
+--+--+ +-----+-	4604,4613 = 4613,4614
+--+--+ +-------	4604,4608 = 4608,4614
+--+--- ++------	4604,4605 = 4604,4615N = 4605,4614 = 4614,4615N
+--+--- +-+-----	4604,4609 = 4609,4614
+--+--- +--+----	4604,4610 = 4610,4614
+--+--- +---+--+	4604,4622 = 4614,4622
+--+--- +----+--	4604,4612 = 4612,4614
+--+--- +-----+-	460103,4604 = 460103,4614
+--+--- +------+	4604,4617 = 4614,4617
+--+--- +-------	4601,4604 = 4601,4614 = 4604,4614 = 4614,4614
+---++-+ ++------	4605,4606 = 4606,4615N
+---++-+ +--+----	4606,4610 = 4619,4620
+---++-+ +-----+-	460103,4606 = 4613,4620
+---++-+ +-------	4601,4606 = 4606,4620 = 4608,4620
+---++-+ --------	4606,4606 = 4606,4608
+---++-- ++------	4605,4620 = 4615N,4620
+---++-- +-------	4601,4620 = 4620,4620
+---+-+- ++------	4605,4607N = 4607N,4615N
+---+-+- +-------	4601,4607N = 4607N,4607N
+---+--+ ++-+----	4605,4619 = 4615N,4619
+---+--+ ++--+-+-	4605,4611 = 4611,4615N

```
+---+--+ ++----+-     4605,4613 = 4613,4615N
+---+--+ ++------     4605,4608 = 4608,4615N
+---+--+ +--+--+-     460103,4619 = 4610,4613
+---+--+ +--+----     4601,4619 = 4608,4610 = 4610,4619
+---+--+ +---+-++     4611,4617 = 4611,4622 = 4613,4622
+---+--+ +---+-+-     4601,4611 = 460103,4611
+---+--+ +-----+-     4601,4613 = 460103,4608 = 460103,4613
+---+--+ ---+----     4608,4619 = 4619,4619
+---+--+ ----+-+-     4608,4611 = 4611,4611 = 4611,4613
+---+--+ ------+-     4608,4613 = 4613,4613
+---+--- ++-----      4605,4609 = 4609,4615N
+---+--- ++-+----      4605,4610 = 4610,4615N
+---+--- ++--+--+     4605,4622 = 4615N,4622
+---+--- ++---+--     4605,4612 = 4612,4615N
+---+--- ++----+-     460103,4605 = 460103,4615N
+---+--- ++-----+     4605,4617 = 4615N,4617
+---+--- ++------     4601,4605 = 4601,4615N = 4605,4615N =
                     4615N,4615N
+---+--- +-+-----     4601,4609 = 4609,4609
+---+--- +--+----     4601,4610 = 4610,4610
+---+--- +---+--+     4601,4622 = 4617,4622 = 4622,4622
+---+--- +----+--     4601,4612 = 4612,4612
+---+--- +-----+-     4601,460103 = 460103,460103
+---+--- +------+     4601,4617 = 4617,4617

4601 = 460101-460102
4613 = 461301-361302
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SPECIFICITY TABLE

HLA-B*46 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-B*46 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified HLA-B*46 alleles	Other amplified HLA Class I alleles[3]
1[4]	130 bp	**800 bp**	*460101-4603, 4605-4622	*1557[W]
2[4]	115 bp	1070 bp	*4602, 4616	
3	395 bp	1070 bp	*4603, 4618, 4621	*1542, 1544, 1550, 1569, 1586, 1593, 9521, 9586
4[4,6]	130 bp, 245 bp	1070 bp	*4604, 4614	*1806, 4073, 7301[W]
5	235 bp	1070 bp	*460101-4604, 4606-4622	*0815, 1557[W], 3574, 4073, 5503
6[4,7]	120 bp, 225 bp	**800 bp**	*4606, 4620	*0709, 0711, 0717, 9538, 1835, 3566, 4038, 4052, 4059, 4060, 4814, 5519, **Cw*0738**
7[4,5,8]	105 bp, 140 bp	1070 bp	*4607N, 4616	*1407N, 3940N, 5619N
8	375 bp	**800 bp**	*4606, 4608, 4611, 461301, 461302, 4618, 4619, 4621	*150201-150303, 150501-1506, 1509-151002, 1513, 1516-151804, 1521, 1523, 152501, 152502, 1529, 1531, 1536, 1537, 1539, 1540, 1542, 1544, 1548, 1552, 1555, 1561, 1562, 1564, 1567, 1569, 1572, 1574, 1580, 1586, 1588-1591, 1593, 1595, 1598, 9503, 9506-9508, 9512, 9514, 9515, 9519, 9521, 9523, 9524, 9527, 9531-9534, 9536, 9538, 9539, 9551, 9553, 9555, 9556, 9558, 9561, 9562, 9568, 9570, 9573, 9576, 9577, 9585, 9586
9[4]	115 bp	1070 bp	*460101-4602, 4604, 4605, 4607N, 4609, 4610, 4612, 4614-4617, 4620, 4622	*1331, 15010101-150104, 150106-150116, 1504, 1507, 1508, 151101-1512, 1514, 1515, 1519, 1524, 1526N-1528, 1530, 1532, 1534, 1535, 153801, 153802, 1543, 1545, 1546, 1550, 1553, 1554, 1556-1558, 1560, 1563, 1566, 1568,

				1570, 1571, 1573, 1575-1577, 1579N, 1581, 1582, 1585, 1587, 1592, 1594N, 1596, 1597, 9501, 9502, 9504, 9505, 9509-9511N, 9513, 9517, 9518, 9520, 9522, 9525, 9526, 9528, 9529, 9535, 9537, 9540, 9542-9549N, 9552, 9554, 9557, 9559, 9560, 9563-9567, 9569, 9571, 9572, 9574, 9575, 9578, 9581N-9584, 1819, 2725, 351401, 351402, 3543, 3544, 3562, 3567, 3579, 3586, 3936, 5161, 5406, 5521, 5603, **Cw*0620**
10[4,5,9]	100 bp, 315 bp	**800 bp**	*4605, 4615N	*9525, 3554
11	150 bp	1070 bp	*4609	*3562, **Cw*0228**
12[5,10]	170 bp, 380 bp	1070 bp	*4610, 4619	*1548, 9508, 9536
13[4,5,11]	105 bp, 215 bp	1070 bp	*4611, 4618, 4622	*0778, 130201-1303, 1308Q, 1309, 1314-1316, 1318, 1319, 1327, 1330-1334, 1542, 3560, 4415, 4418, 4501, 4503-4508, 4510, 4511, 490101-4903, 4906-4909, 500101-5002, 5004-5008, 5115, 5401-5403, 5405N, 5407, 5408N, 5410, 5412, 5413, 5416-5419, 550101-550103, 550105-5503, 5505, 5507, 5509-5512, 5515, 5516, 5518, 5519, 5521, 5522, 5524-5526, 5529-5531, 5533-5538, 560101-560103, 5607, 5608, 5613, 5614, 5616, 5617, 5619N, 5620, 5623-5627, 5901, 5904, 5905
14[5]	215 bp	1070 bp	*4612	*0755, 1507, 1545, 1568, 9526, 4819
15	325 bp	1070 bp	*460103, 4611, 461301, 461302, 4618	*150302, 150502, 152703, 153802, 1542, 1548, 1586
16[5]	205 bp	1070 bp	*4617, 4622	*1514, 1591, 9531, 9561, 3545, 3571, 4417, 4443, 4509, 5807

[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*46 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 lengths 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*46 SSP subtyping. In addition, well number 6, 8 and 10 contains the primer pair giving rise to the longer, 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 one non-HLA-B*46 alleles will be amplified by all primer mixes except for mix 2. In addition, the Cw*0738 allele will be amplified by primer mix 6, the Cw*0620 allele will be amplified by primer mix 9 and the Cw*0228 allele will be amplified by primer mix 11.
[4]Specific PCR fragments shorter than 150 base pairs are less intense and not as sharp as longer specific bands.
[5]Primer mixes 7, 10, 13, 14 and 16 may give rise to nonspecific amplifications.
[6]Primer mix 4: Specific PCR fragment of 130 bp in the B*4604 allele. Specific PCR fragment of 245 bp in the B*4614 and in the B*1806, 4073 and 7301[w] alleles.
[7]Primer mix 6: Specific PCR fragment of 120 bp in the B*4620 and the B*5519 alleles. Specific PCR fragment of 225 bp in the B*4606 and in the B*0709, 0711, 0717, 9538, 1835, 3566, 4038, 4052, 4059, 4060, 4814 and Cw*0738 alleles.
[8]Primer mix 7: Specific PCR fragment of 105 bp in the B*4616 allele. Specific PCR fragment of 140 bp in the B*4607N and in the B*1407N, 3940N and 5619N alleles.
[9]Primer mix 10: Specific PCR fragment of 100 bp in the B*4605 and in the B*9525 and 3554 alleles. Specific PCR fragment of 315 bp in the B*4615N allele.
[10]Primer mix 12: Specific PCR fragment of 170 bp in the B*4610 allele. Specific PCR fragment of 380 bp in the B*4619 and in the B*1548, 9508 and 9536 alleles.
[11]Primer mix 13: Specific PCR fragment of 105 bp in the B*4611, 4618 and in the B*0778, 130201-1303, 1308Q, 1309, 1314-1316, 1318, 1319, 1327, 1330-1334, 1542, 3560, 4415, 4418, 4501, 4503-4508, 4510, 4511, 490101-4903, 4906-4909, 500101-5002, 5004-5008, 5115, 5401-5403, 5405N, 5407, 5408N, 5410, 5412, 5413, 5416-5419, 550101-550103, 550105-5503, 5505, 5507, 5509-5512, 5515, 5516, 5518, 5519, 5521, 5522, 5524-5526, 5529-5531, 5533-5538, 560101-560103, 5607, 5608, 5613, 5614, 5616, 5617, 5619N, 5620, 5623-5627, 5901, 5904 and 5905 alleles. Specific PCR fragment of 215 bp in the B*4622 allele.
'w', might be weakly amplified.

INTERPRETATION TABLE

HLA-B*46 SSP subtyping

Amplification patterns of the B*4601 to 4622 alleles

	Well[4]							
	1	2	3	4	5	6	7	8
Length of spec.	130	115	395	130	235	120	105	375
PCR product(s)				245		225	140	
Length of int.	800	1070	1070	1070	1070	800	1070	800
pos. control[1]								
5'-primer(s)[2]	209	209	2nd I	97	106	106	209	2nd I
	5' -ggC 3'	5' -ggC 3'	5' -CAA 3'	5' -TCC 3'	5' -CCA 3'	5' -CCA 3'	5' -ggC 3'	5' -CAA 3'
				209		419	463	
				5' -ggg 3'		5' -gTC 3'	5' -TgA 3'	
3'-primer(s)[3]	299	272	559	299	299	187	272	538
	5' -TCA 3'	5' -TgA 3'	5' -CgT 3'	5' -TCA 3'	5' -TCA 3'	5' -gTT 3'	5' -TgA 3'	5' -CAg 3'
		293				605	564	544
		5' -ggC 3'				5' -gCT 3'	5' -ACT 3'	5' -ggT 3'
Well No.	1	2	3	4	5	6	7	8
HLA-B allele								
***460101, 460102**	1				5			
***460103**	1				5			
***4602**	1	2			5			
***4603**	1		3		5			
***4604**				4	5			
***4605**	1							
***4606**	1				5	6		8
***4607N**	1				5		7	
***4608**	1				5			8
***4609**	1				5			
***4610**	1				5			
***4611**	1				5			8
***4612**	1				5			
***461301, 461302**	1				5			8
***4614**	1			4	5			
***4615N**	1				5			
***4616**	1	2			5		7	
***4617**	1				5			
***4618**	1		3		5			8
***4619**	1				5			8
***4620**	1				5	6		
***4621**	1		3		5			8
Well No.	1	2	3	4	5	6	7	8

INTERPRETATION TABLE

HLA-B*46 SSP subtyping

Amplification patterns of the B*4601 to 4622 alleles

Well[4]								
9	10	11	12	13	14	15	16	
115	100	150	170	105	215	325	205	Length of spec.
	315		380	215				PCR product(s)
1070	800	1070	1070	1070	1070	1070	1070	Length of int.
								pos. control[1]
463	106	419	142	106	363	2nd I	106	5'-primer(s)[2]
5' -TgA 3'	5' -CCg 3'	5' -gTC 3'	5' -TCA 3'	5' -CCA 3'	5' -AgC 3'	5' -CAA 3'	5' -CCA 3'	
	736		2nd I	357			419	
	5' -gCT 3'		5' -CAA 3'	5' -Tgg 3'			5' -gTC 3'	
538	165	527	269	281	538	486	281	3'-primer(s)[3]
5' -CCA 3'	5' -Tgg 3'	5' -CCA 3'	5' -ACT 3'	5' -CCC 3'	5' -CCA 3'	5' -gCg 3'	5' -CCC 3'	
	916		544	420		498	572	
	5' -gAT 3'		5' -ggT 3'	5' -gCT 3'		5' -gTA 3'	5' -gCg 3'	
9	10	11	12	13	14	15	16	Well No.
								HLA-B allele
9								*460101, 460102
9						15		*460103
9								*4602
								*4603
9								*4604
9	10							*4605
								*4606
9								*4607N
								*4608
9		11						*4609
9			12					*4610
				13		15		*4611
9					14			*4612
						15		*461301, 461302
9								*4614
9	10							*4615N
9								*4616
9							16	*4617
				13		15		*4618
			12					*4619
9								*4620
								*4621
9	10	11	12	13	14	15	16	Well No.

Length of spec.	**130**	**115**	**395**	**130**	**235**	**120**	**105**	**375**
PCR product(s)				**245**		**225**	**140**	
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
***4622**	**1**				**5**			
0709, 0711, 0717, 1835, 3566, 4038, 4052, 4059, 4060, 4814, *Cw*0738						**6**		
***0755, 4819**								
***0778, 130201-1303, 1308Q, 1309, 1314-1316, 1318, 1319, 1327, 1330, 1332-1334, 3560, 4415, 4418, 4501, 4503-4508, 4510, 4511, 490101-4903, 4906-4909, 500101-5002, 5004-5008, 5115, 5401-5403, 5405N, 5407, 5408N, 5410, 5412, 5413, 5416-5419, 550101-550103, 550105-550205, 5505, 5507, 5509-5512, 5515, 5516, 5518, 5522, 5524-5526, 5529-5531, 5533-5538, 560101-560103, 5607, 5608, 5613, 5614, 5616, 5617, 5620, 5623-5627, 5901, 5904, 5905**								
***0815, 3574**					**5**			
***1331, 5521**								
***1407N, 3940N**							**7**	
15010101-150104, 150106-150116, 1504, 1508, 151101-1512, 1515, 1519, 1524, 1526N-152702, 1528, 1530, 1532, 1534, 1535, 153801, 1543, 1546, 1553, 1554, 1556, 1558, 1560, 1563, 1566, 1570, 1571, 1573, 1575-1577, 1579N, 1581, 1582, 1585, 1587, 1592, 1594N, 1596, 1597, 9501, 9502, 9504, 9505, 9509-9511N, 9513, 9517, 9518, 9520, 9522, 9528, 9529, 9535, 9537, 9540, 9542-9549N, 9552, 9554, 9557, 9559, 9560, 9563-9567, 9569, 9571, 9572, 9574, 9575, 9578, 9581N-9584, 1819, 2725, 351401, 351402, 3543, 3544, 3567, 3579, 3586, 3936, 5161, 5406, 5603, *Cw*0620								
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**

115	100	150	170	105	215	325	205	Length of spec.
	315		380	215				PCR product(s)
9	10	11	12	13	14	15	16	Well No.
9				13			16	*4622
								*0709, 0711, 0717, 1835, 3566, 4038, 4052, 4059, 4060, 4814, *Cw*0738*
					14			*0755, 4819
				13				*0778, 130201-1303, 1308Q, 1309, 1314-1316, 1318, 1319, 1327, 1330, 1332-1334, 3560, 4415, 4418, 4501, 4503-4508, 4510, 4511, 490101-4903, 4906-4909, 500101-5002, 5004-5008, 5115, 5401-5403, 5405N, 5407, 5408N, 5410, 5412, 5413, 5416-5419, 550101-550103, 550105-550205, 5505, 5507, 5509-5512, 5515, 5516, 5518, 5522, 5524-5526, 5529-5531, 5533-5538, 560101-560103, 5607, 5608, 5613, 5614, 5616, 5617, 5620, 5623-5627, 5901, 5904, 5905
								*0815, 3574
9				13				*1331, 5521
								*1407N, 3940N
9								*15010101-150104, 150106-150116, 1504, 1508, 151101-1512, 1515, 1519, 1524, 1526N-152702, 1528, 1530, 1532, 1534, 1535, 153801, 1543, 1546, 1553, 1554, 1556, 1558, 1560, 1563, 1566, 1570, 1571, 1573, 1575-1577, 1579N, 1581, 1582, 1585, 1587, 1592, 1594N, 1596, 1597, 1819, 2725, 351401, 351402, 3543, 3544, 3567, 3579, 3586, 3936, 5161, 5406, 5603, 9501, 9502, 9504, 9505, 9509-9511N, 9513, 9517, 9518, 9520, 9522, 9528, 9529, 9535, 9537, 9540, 9542-9549N, 9552, 9554, 9557, 9559, 9560, 9563-9567, 9569, 9571, 9572, 9574, 9575, 9578, 9581N-9584, *Cw*0620*
9	10	11	12	13	14	15	16	Well No.

Length of spec.	**130**	**115**	**395**	**130**	**235**	**120**	**105**	**375**
PCR product(s)				**245**		**225**	**140**	
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
***150201-150301, 150303, 150501, 1506, 1509-151002, 1513, 1516-151804, 1521, 1523, 152501, 152502, 1529, 1531, 1536, 1537, 1539, 1540, 1552, 1555, 1561, 1562, 1564, 1567, 1572, 1574, 1580, 1588-1590, 1595, 1598, 9503, 9506, 9507, 9512, 9514, 9515, 9519, 9523, 9524, 9527, 9532-9534, 9539, 9551, 9553, 9555, 9556, 9558, 9562, 9568, 9570, 9573, 9576, 9577, 9585**								**8**
***150302, 150502**								**8**
***1507, 1545, 1568, 9526**								
***1514**								
***152703, 153802**								
***1542**			**3**					**8**
***1544, 1569, 1593, 9521, 9586**			**3**					**8**
***1548**								**8**
***1550**			**3**					
***1557**	**w**				**w**			
***1586**			**3**					**8**
***1591, 9531, 9561**								**8**
***9508, 9536**								**8**
***9525**								
***9538**						**6**		**8**
***1806**				**4**				
***3545, 3571, 4417, 4443, 4509, 5807**								
***3554**								
***3562**								
***4073**				**4**	**5**			
***5503**					**5**			
***5519**						**6**		
***5619N**							**7**	
***7301**				**w**				
Cw*0228								
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**

115	100	150	170	105	215	325	205	Length of spec.
	315		380	215				PCR product(s)
9	10	11	12	13	14	15	16	Well No.
								*150201-150301, 150303, 150501, 1506, 1509-151002, 1513, 1516-151804, 1521, 1523, 152501, 152502, 1529, 1531, 1536, 1537, 1539, 1540, 1552, 1555, 1561, 1562, 1564, 1567, 1572, 1574, 1580, 1588-1590, 1595, 1598, 9503, 9506, 9507, 9512, 9514, 9515, 9519, 9523, 9524, 9527, 9532-9534, 9539, 9551, 9553, 9555, 9556, 9558, 9562, 9568, 9570, 9573, 9576, 9577, 9585
						15		*150302, 150502
9					14			*1507, 1545, 1568, 9526
9							16	*1514
9						15		*152703, 153802
				13		15		*1542
								*1544, 1569, 1593, 9521, 9586
			12			15		*1548
9								*1550
9								*1557
						15		*1586
							16	*1591, 9531, 9561
			12					*9508, 9536
9	10							*9525
								*9538
								*1806
							16	*3545, 3571, 4417, 4443, 4509, 5807
	10							*3554
9		11						*3562
								*4073
				13				*5503
				13				*5519
				13				*5619N
								*7301
		11						*Cw*0228*
9	10	11	12	13	14	15	16	Well No.

[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*46 SSP subtyping.
In addition, wells number 6, 8 and 10 contain the primer pair giving rise to the shorter, 800 bp, internal positive control in order to allow kit identification.
[2]The nucleotide position, in the 2nd, 3rd or 4th exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.
[3]The nucleotide position, in the 2nd, 3rd and 4th exon or in the 2nd 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]Primer mix 4: Specific PCR fragment of 130 bp in the B*4604 allele. Specific PCR fragment of 245 bp in the B*4614 and in the B*1806, 4073 and 7301[w] alleles.
Primer mix 6: Specific PCR fragment of 120 bp in the B*4620 and the B*5519 alleles. Specific PCR fragment of 225 bp in the B*4606 and in the B*0709, 0711, 0717, 9538, 1835, 3566, 4038, 4052, 4059, 4060, 4814 and Cw*0738 alleles.
Primer mix 7: Specific PCR fragment of 105 bp in the B*4616 allele. Specific PCR fragment of 140 bp in the B*4607N and in the B*1407N, 3940N and 5619N alleles.
Primer mix 10: Specific PCR fragment of 100 bp in the B*4605 and in the B*9525 and 3554 alleles. Specific PCR fragment of 315 bp in the B*4615N allele.
Primer mix 12: Specific PCR fragment of 170 bp in the B*4610 allele. Specific PCR fragment of 380 bp in the B*4619 and in the B*1548, 9508 and 9536 alleles.
Primer mix 13: Specific PCR fragment of 105 bp in the B*4611, 4618 and in the B*0778, 130201-1303, 1308Q, 1309, 1314-1316, 1318, 1319, 1327, 1330-1334, 1542, 3560, 4415, 4418, 4501, 4503-4508, 4510, 4511, 490101-4903, 4906-4909, 500101-5002, 5004-5008, 5115, 5401-5403, 5405N, 5407, 5408N, 5410, 5412, 5413, 5416-5419, 550101-550103, 550105-5503, 5505, 5507, 5509-5512, 5515, 5516, 5518, 5519, 5521, 5522, 5524-5526, 5529-5531, 5533-5538, 560101-560103, 5607, 5608, 5613, 5614, 5616, 5617, 5619N, 5620, 5623-5627, 5901, 5904 and 5905 alleles. Specific PCR fragment of 215 bp in the B*4622 allele.
'w', might be weakly amplified.

CELL LINE VALIDATION SHEET

HLA-B*46 SSP primer set

					Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.:	200623201	200956602	200956603	200956604	200623205	201069306	200956607	201069308	200623209	200956610	200737911	201069312	201069313	200737914	201069315	201069316
	IHWC cell line		B*																	
1	9001	SA	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*5201	*7301	-	-	-	w	-	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*52011		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*1510	*5301	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
5	9009	KAS011	*3701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*3901	*5101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*1801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025	DEU	*3501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*5401		-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
11	9051	PITOUT	*4403		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*5701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*2705		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*1501	*1520	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
15	9075	DKB	*4001		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*4002		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*0801	*5501	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
18	9257	32367	*1401	*5601	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
19	9038	BM16	*1801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*4001		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*1501		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
22	9056	KOSE	*3503		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*4002	*5602	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*1402		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*5801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*0705	*5101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*4402	*4403	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*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*46 SSP

Product number: **101.544-06u – without *Taq* polymerase**
Lot number: **81G**
Expiry date: **2012-February-01**
Number of tests: **6**
Number of wells per test: **16**

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2006-232-01	9	2006-232-09
2	2009-566-02	10	2009-566-10
3	2009-566-03	11	2007-379-11
4	2009-566-04	12	2010-693-12
5	2006-232-05	13	2010-693-13
6	2010-693-06	14	2007-379-14
7	2009-566-07	15	2010-693-15
8	2010-693-08	16	2010-693-16

The specificity of each primer solution of the HLA-B*46 primer set has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 2 to 4, 6, 7, 10 to 12 and 15 were available. The specificities of the primers in primer solutions 3, 4, 6, 10 to 12 and 15 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 2, 5, 9 and 13 it was only possible to test the 5'-primer, the 3'-primer was not possible to test.
In primer solutions 2 and 7 it was only possible to the 3'-primer, the 5'-primer was not possible to test. In primer solutions 4 and 10 one of the 5'-primers was not possible to test, and in primer solutions 6, 8 and 13 to 16 one of the 5'-primers was not possible to test. Additional 5'-primers in primer solutions 13 and 15 were tested by separately adding one 3'-primer.

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

Date of approval: 2010-February-12

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*46
Product number: 101.544-06u
Lot number: 81G

Intended use: HLA-B*46 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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Phone: +46-8-717 88 27
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We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2008 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, conformity assessed using Annex IV, as transposed into the national laws of the Member States of the European Union.

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

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

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

Saltsjöbaden, Sweden
2010-February-12

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