Olerup SSP® DRB1*12

Product number:	**101.128-12u – without *Taq* polymerase**
Lot number:	**83M**
Expiry date:	**2014-April-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. 83M.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® DRB1*12 LOT

The DRB1*12 specificity and interpretation tables have been updated for the DRB1 alleles described since the previous *Olerup* SSP® DRB1*12 lot was made **(Lot No. 51K)**.

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

Well	5'-primer	3'-primer	rationale
17	-	Modified	3'-primer modified for improved amplification of the DRB1*12:10 allele.
20	Added	-	Primer added for resolution of the DRB1*12:27 allele.

PRODUCT DESCRIPTION

DRB1*12 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*12:01 to DRB1*12:27 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 'DRB1*12' in silver/gray ink.
Well No. 1 is marked with the Lot No. '83M'.
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 covered with a PCR-compatible foil.
Please note: When removing each 24 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 DRB1*12 SSP subtypings will be influenced by the DRB1*01, most DRB1*08, two DRB1*11, four DRB1*13 and fourteen DRB1*14 alleles when present on the other haplotype. In addition, the DRB1*15 and DRB1*16 alleles will be weakly amplified by primer mix 14.

UNIQUELY IDENTIFIED ALLELES

All the phenotypically different DRB1*12 alleles, i.e. **DRB1*12:01 to DRB1*12:27**, recognized by the HLA Nomenclature Committee in April 2011[1] will give rise to unique amplification patterns by the primers in the DRB1*12 subtyping kit.
The DRB1*12 kit cannot distinguish the DRB1*12:01:02-12:01:03 alleles or the DRB1*12:02:01 and 12:02:03-12:02:05 alleles.

[1]DRB1 alleles listed on the IMGT/HLA web page 2011-April-08, release 3.4.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 41 alleles generate 29 amplification patterns that can be combined in 435 homozygous and heterozygous combinations. 260 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products were not considered in these calculations.

++++++++ ++--+--- -----+---	*12:09, *12:15 = *12:09, *12:20
++++++++ ++------ -----++-	*12:09, *12:16 = *12:09, *12:21
++++++++ ++------ -----+--	*12:02:01, *12:09 = *12:02:02, *12:09
++++++++ +---+--- -----+--	*12:05, *12:09 = *12:09, *12:14 = *12:09, *12:24N
++++++++ +------- -----+--	*12:01:01, *12:09 = *12:01:02, *12:09
++-++++ +++-+--- -----+--	*12:03:02, *12:20 = *12:05, *12:19 = *12:14, *12:19 = *12:19, *12:24N
++-++++ ++------ -+--+---	*12:03:02, *12:26 = *12:11, *12:19
++-++++ ++------ -----+--	*12:01:01, *12:19 = *12:01:02, *12:19 = *12:02:01, *12:03:02
++-++++ ++-+--- -----+--	*12:04, *12:15 = *12:04, *12:20
++-++++ ++-+--- -----++-	*12:04, *12:16 = *12:04, *12:21
++-++++ ++-+--- -----+--	*12:02:01, *12:04 = *12:02:02, *12:04
++-++++ ++--+--- -----+--	*12:06, *12:15 = *12:06, *12:20
++-++++ ++---+-- -----+--	*12:05, *12:23 = *12:14, *12:23 = *12:23, *12:24N
++-++++ ++---+-- -----+--	*12:05, *12:27 = *12:08, *12:15 = *12:08, *12:20 = *12:14, *12:27 = *12:24N, *12:27
++-++++ ++--+--- +---+-+-	*12:15, *12:25 = *12:20, *12:25
++-++++ ++--+--- +---+---	*12:10, *12:15 = *12:10, *12:20
++-++++ ++--+--- -+--+---	*12:05, *12:26 = *12:11, *12:15 = *12:11, *12:20 = *12:14, *12:26 = *12:24N, *12:26
++-++++ ++--+--- -----+--	*12:05, *12:13 = *12:13, *12:14 = *12:13, *12:24N
++-++++ ++--+--- -----++-	*12:05, *12:21 = *12:14, *12:21 = *12:16, *12:24N = *12:20, *12:22 = *12:21, *12:24N
++-++++ ++--+--- -----+-+-	*12:15, *12:17 = *12:17, *12:20
++-++++ ++--+--- ------+	*12:05, *12:18 = *12:14, *12:18 = *12:18, *12:24N
++-++++ ++--+--- -----+--	*12:01:01, *12:15 = *12:01:01, *12:20 = *12:01:02, *12:20 = *12:02:01, *12:05 = *12:02:01, *12:14 = *12:02:01, *12:24N = *12:02:02, *12:24N = *12:05, *12:20 = *12:14, *12:20 = *12:15, *12:24N = *12:20, *12:24N
++-++++ ++---+-- -----++-	*12:06, *12:16 = *12:06, *12:21
++-++++ ++---+-- -----+--	*12:02:01, *12:06 = *12:02:02, *12:06
++-++++ ++-----+ -+--+---	*12:08, *12:26 = *12:11, *12:27
++-++++ ++-----+ -----+--	*12:01:01, *12:23 = *12:01:02, *12:23 = *12:08, *12:13
++-++++ ++-----+ -----+--	*12:01:01, *12:27 = *12:01:02, *12:27 = *12:02:01, *12:08 = *12:02:02, *12:08
++-++++ ++------ +-----+-	*12:16, *12:25 = *12:21, *12:25
++-++++ ++------ +-----+-	*12:10, *12:16 = *12:10, *12:21
++-++++ ++------ +----+-+-	*12:02:01, *12:25 = *12:02:02, *12:25
++-++++ ++------ +----+--	*12:02:01, *12:10 = *12:02:02, *12:10
++-++++ ++------ -+--+--	*12:11, *12:16 = *12:11, *12:21 = *12:22, *12:26
++-++++ ++------ -+--+---	*12:01:01, *12:26 = *12:01:02, *12:26 = *12:02:01, *12:11 = *12:02:02, *12:11 = *12:11, *12:26
++-++++ ++------ ---+---	*12:01:01, *12:13 = *12:01:02, *12:13
++-++++ ++------ -----++-	*12:16, *12:17 = *12:17, *12:21
++-++++ ++------ -----+--	*12:01:01, *12:16 = *12:01:01, *12:21 = *12:01:02, *12:21 = *12:02:01, *12:22 = *12:21, *12:22
++-++++ ++------ -----+-+-	*12:02:01, *12:17 = *12:02:02, *12:17
++-++++ ++------ ------+	*12:01:01, *12:18 = *12:01:02, *12:18
++-++++ ++------ -----+--	*12:01:01, *12:02:01 = *12:01:01, *12:02:02 = *12:01:02, *12:02:01
++-++++ +--+--- -----+--	*12:04, *12:05 = *12:04, *12:14 = *12:04, *12:24N
++-++++ +--+--- -----+--	*12:01:01, *12:04 = *12:01:02, *12:04
++-++++ +---+-- -----+--	*12:05, *12:06 = *12:06, *12:14 = *12:06, *12:24N
++-++++ +---+-+- -----+--	*12:05, *12:07 = *12:07, *12:14 = *12:07, *12:24N

++-+++++ +---+--+ ----+---	*12:05, *12:08 = *12:08, *12:14 = *12:08, *12:24N
++-+++++ +---+--- +----+-	*12:05, *12:25 = *12:14, *12:25 = *12:24N, *12:25
++-+++++ +---+--- +---+---	*12:05, *12:10 = *12:10, *12:14 = *12:10, *12:24N
++-+++++ +---+--- -+--+---	*12:05, *12:11 = *12:11, *12:14 = *12:11, *12:24N
++-+++++ +---+--- --+-+---	*12:05, *12:12 = *12:12, *12:14 = *12:12, *12:24N
++-+++++ +---+--- -----+-	*12:05, *12:17 = *12:14, *12:17 = *12:17, *12:24N
++-+++++ +---+--- ----+---	*12:01:01, *12:05 = *12:01:01, *12:14 = *12:01:01, *12:24N = *12:01:02, *12:24N = *12:05, *12:24N = *12:14, *12:24N = *12:24N, *12:24N
++-+++++ +----+-- ----+---	*12:01:01, *12:06 = *12:01:02, *12:06 = *12:06, *12:06
++-+++++ +-----+- ----+---	*12:01:01, *12:07 = *12:01:02, *12:07
++-+++++ +------+ ----+---	*12:01:01, *12:08 = *12:01:02, *12:08
++-+++++ +------- +----+-	*12:01:01, *12:25 = *12:01:02, *12:25 = *12:10, *12:17 = *12:10, *12:25 = *12:17, *12:25 = *12:25, *12:25
++-+++++ +------- +---+---	*12:01:01, *12:10 = *12:01:02, *12:10 = *12:10, *12:10
++-+++++ +------- -+--+---	*12:01:01, *12:11 = *12:01:02, *12:11 = *12:11, *12:11
++-+++++ +------- --+-+---	*12:01:01, *12:12 = *12:01:02, *12:12
++-+++++ +------- -----+-	*12:01:01, *12:17 = *12:01:02, *12:17 = *12:17, *12:17
++-+++++ +------- ----+---	*12:01:01, *12:01:01 = *12:01:01, *12:01:02
++-+++++ -+--+--- ----+--	*12:05, *12:16 = *12:14, *12:16 = *12:15, *12:22
++-+++++ -+--+--- ----+---	*12:01:02, *12:15 = *12:02:02, *12:05 = *12:02:02, *12:14
++-+++++ -+------ ----+--	*12:01:02, *12:16 = *12:02:02, *12:22
++-+++++ --+-+--- ----+---	*12:03:02, *12:05 = *12:03:02, *12:14
++-+++++ ----+--- ----+--	*12:05, *12:22 = *12:14, *12:22
++-+++++ ----+--- ----+---	*12:01:02, *12:05 = *12:01:02, *12:14
++-++++- +++-+--- ----+---	*12:15, *12:19 = *12:19, *12:20
++-++++- +++----- ----+---	*12:02:01, *12:19 = *12:02:02, *12:19
++-++++- ++--+-+- ----+---	*12:07, *12:15 = *12:07, *12:20
++-++++- ++--+--+ ----+---	*12:15, *12:23 = *12:20, *12:23
++-++++- ++--+--+ ----+---	*12:15, *12:27 = *12:20, *12:27
++-++++- ++--+--- -+--+---	*12:15, *12:26 = *12:20, *12:26
++-++++- ++--+--- --+-+---	*12:12, *12:15 = *12:12, *12:20
++-++++- ++--+--- ---++---	*12:13, *12:15 = *12:13, *12:20
++-++++- ++--+--- ----+--	*12:15, *12:21 = *12:16, *12:20 = *12:20, *12:21
++-++++- ++--+--- ----+--+	*12:15, *12:18 = *12:18, *12:20
++-++++- ++--+--- ----+---	*12:02:01, *12:15 = *12:02:01, *12:20 = *12:02:02, *12:20 = *12:15, *12:20 = *12:20, *12:20
++-++++- ++----+- ----++--	*12:07, *12:16 = *12:07, *12:21
++-++++- ++----+- ----+---	*12:02:01, *12:07 = *12:02:02, *12:07
++-++++- ++-----+ ----+---	*12:02:01, *12:23 = *12:02:02, *12:23 = *12:13, *12:23 = *12:13, *12:27
++-++++- ++-----+ ----+---	*12:02:01, *12:27 = *12:02:02, *12:27
++-++++- ++------ -+--++--	*12:16, *12:26 = *12:21, *12:26
++-++++- ++------ -+--+---	*12:02:01, *12:26 = *12:02:02, *12:26 = *12:26, *12:26
++-++++- ++------ --+-++--	*12:12, *12:16 = *12:12, *12:21
++-++++- ++------ --+-+---	*12:02:01, *12:12 = *12:02:02, *12:12
++-++++- ++------ ---+++--	*12:13, *12:16 = *12:13, *12:21
++-++++- ++------ ---++---	*12:02:01, *12:13 = *12:02:02, *12:13 = *12:13, *12:13
++-++++- ++------ ----++-+	*12:16, *12:18 = *12:18, *12:21
++-++++- ++------ ----++--	*12:02:01, *12:16 = *12:02:01, *12:21 = *12:02:02, *12:21 = *12:16, *12:21
++-++++- ++------ -----+--	*12:02:01, *12:18 = *12:02:02, *12:18
++-++++- ++------ ----+---	*12:02:01, *12:02:01 = *12:02:01, *12:02:02
++-+++-+ +++----+ ----+---	*12:03:02, *12:27 = *12:08, *12:19
++-+++-+ ++-----+ ----++--	*12:08, *12:16 = *12:22, *12:27
++-+++-- ++-----+ ----+---	*12:23, *12:23 = *12:23, *12:27
++--++++ -+--+--- ----+---	*12:05, *12:15 = *12:14, *12:15
++--++++ ----+--- ----+---	*12:05, *12:05 = *12:05, *12:14

*12:01:02 = *12:01:02-12:01:03, *12:02:01 = *12:02:01, 12:02:03-12:02:05

SPECIFICITY TABLE

DRB1*12 SSP subtyping

Specificities and sizes of the PCR products of the 24 primer mixes used for DRB1*12 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified DRB1*12 alleles[3]	Other amplified DRB1 alleles[4]
1	135 bp	**515 bp**	*12:01:01-12:20, 12:22-12:27	*08:17, 08:28, 08:37, 08:45, 11:67, 13:17
2	215 bp	430 bp	*12:01:01-12:21, 12:23-12:27	*08:05, 08:18, 08:24-08:25, 08:31, 08:40-08:41, 13:17, 13:116, 14:31, 14:52
3	165 bp	430 bp	*12:09	*08:02:01-08:02:03, 08:04:01-08:04:05, 08:04:07, 08:09, 08:13, 08:21, 08:24, 08:28, 08:30:01, 08:42, 08:44-08:45, 13:17, 13:116, 14:15, 14:52
4[5]	105 bp	430 bp	*12:01:01-12:04, 12:06-12:13, 12:16-12:27	*08:32
5	165 bp	**515 bp**	*12:01:01-12:03:02, 12:05-12:08, 12:10-12:17, 12:19-12:23, 12:24N[W], 12:25-12:27	*08:19, 08:25, 08:34
6	250 bp	430 bp	*12:01:01-12:02:05, 12:04-12:15, 12:17-12:18, 12:20-12:21, 12:23-12:27	*08:12, 08:22, 14:28
7	215 bp	430 bp	*12:01:01-12:02:05, 12:04-12:07, 12:09-12:12, 12:13[W], 12:14-12:15, 12:17-12:18, 12:20-12:21, 12:24N-12:26	
8	195 bp	430 bp	*12:01:01-12:01:03, 12:03:02-12:06, 12:08-12:11, 12:14, 12:17, 12:22, 12:24N-12:25	*08:03:02, 08:10, 08:12, 08:14-08:15, 08:18-08:19, 08:23, 08:25, 08:27, 08:29-08:30:02, 08:32-08:38, 08:40, 08:45

9	165 bp	430 bp	*12:01:01, 12:02:01, 12:02:03-12:02:05, 12:04, 12:06-12:13, 12:17-12:21, 12:23-12:27	
10	195 bp	430 bp	*12:02:01-12:02:05, 12:13, 12:15-12:16, 12:18-12:21, 12:23, 12:26-12:27	*08:01:01-08:02:03, 08:04:01-08:09, 08:11, 08:16-08:17, 08:21-08:22, 08:24, 08:26, 08:28, 08:31, 08:39, 08:41-08:44, 11:67, 14:15, 14:73
11	250 bp	430 bp	*12:03:02, 12:19	*08:04:01, 08:04:02^{W}-08:04:03^{W}, 08:04:04-08:04:07, 08:06, 08:10, 08:28, 08:31, 11:67, 13:17, 13:116, 14:04, 14:11, 14:15, 14:31, 14:50, 14:52, 14:73, 14:76, 14:79, 14:107
12[6]	170 bp	430 bp	*12:04	*08:31, 08:41, 11:67, 14:11
13[5,9]	115 bp, 185 bp, 255 bp	**515 bp**	*12:05, 12:14-12:15, 12:20, 12:24N	
14[6,8]	135 bp	**515 bp**	*12:06	*15:01:01:01^{W}-15:01:16^{W}, 15:02:01^{W}-15:57^{W}, 16:01:01^{W}-16:05:02^{W}, 16:07^{W}-16:18^{W}
15	200 bp	**515 bp**	*12:07	
16[5,7]	75 bp	430 bp	*12:08, 12:23, 12:27	*11:76, 13:34, 13:64, 14:41, 14:77, 14:110
17[5,7]	90 bp	430 bp	*12:10, 12:25	
18[5,10]	90 bp, 135 bp	**515 bp**	*12:11, 12:26	
19	195 bp	430 bp	*12:12	*08:13
20	220 bp	430 bp	*12:13, 12:23	
21[5]	105 bp	430 bp	*12:01:01-12:13, 12:15-12:27	*08:32
22[5,11]	105 bp, 220 bp	430 bp	*12:16, 12:21-12:22	*08:32
23[5]	110 bp	430 bp	*12:17, 12:25	*01:01:01-01:36
24	170 bp	430 bp	*12:18	

[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 DRB1*12 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 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.
Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB1*12 subtyping.
In addition, wells number 5, 13 to 15 and 18 contain the primer pair giving rise to the longer, 515 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]For several DRB alleles only partial second exon and third exon nucleotide sequences are available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. We assume that unknown sequences in the first hyperpolymorphic region of the second exon of DRB alleles are conserved within allelic groups and that unknown sequences of codons 87 to 92 are identical with the DRB1*01:01 consensus sequence.
[4]Due to the sharing of sequence motifs, non-DRB1*12 alleles will be amplified by primer mixes 1 to 6, 8, 10 to 12, 16, 19 and 21 to 23. In addition, the DRB1*15 and DRB1*16 alleles will be weakly amplified by primer mix 14.
[5]Short specific PCR fragments have a lower intensity than longer PCR bands.
[6]Primer mixes 12 and 14 may give rise to a primer dimer artefact.
[7]Primer mixes 16 and 17 may have tendencies of giving rise to unspecific amplifications.
[8]Primer mix 14 may give a lower yield of HLA-specific PCR product than the other DRB1*12 primer mixes.
[9]Primer mix 13: Specific PCR fragment of 115 bp in the DRB1*12:24N allele. Specific PCR fragment of 185 bp in the DRB1*12:05, 12:14 and 12:15 alleles. Specific PCR fragment of 255 bp in the DRB1*12:20 allele.
[10]Primer mix 18: Specific PCR fragment of 90 bp in the DRB1*12:26 allele. Specific PCR fragment of 135 bp in the DRB1*12:11 allele.
[11]Primer mix 22: Specific PCR fragment of 105 bp in the DRB1*12:21 allele. Specific PCR fragment of 220 bp in the DRB1*12:16 and the DRB1*12:22 alleles. Specific PCR fragment of 105 and 220 bp in the DRB1*08:32 allele.
'w', may be weakly amplified.

INTERPRETATION TABLE

DRB1*12 SSP subtyping

Amplification patterns of the DRB1*12:01 to 12:27 alleles

	Well[5]											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Length of spec.	**135**	**215**	**165**	**105**	**165**	**250**	**215**	**195**	**165**	**195**	**250**	**170**
PCR product(s)												
Length of int.	**515**	**430**	**430**	**430**	**515**	**430**	**430**	**430**	**430**	**430**	**430**	**430**
pos. control[1]												
5'-primer[2]	**16 (133)** 5'-gTT-3'	**16 (133)** 5'-gTT-3'	**16 (133)** 5'-gTT-3'	**16 (133)** 5'-gTT-3'	**16 (133)** 5'-gTT-3'	**16 (133)** 5'-gTT-3'	**26 (165)** 5'-TTA-3'	**16 (133)** 5'-gTT-3'	**37 (196)** 5'-AgC-3'	**16 (133)** 5'-gTT-3'	**16 (133)** 5'-gTT-3'	**16 (133)** 5'-gTT-3'
3'-primer[3]	**47 (227)** 5'-ggA-3'	**74 (307)** 5'-CgC-3'	**57 (257)** 5'-CAT-3'	**37 (196)** 5'-gAg-3'	**57 (257)** 5'-CgA-3'	**85 (341)** 5'-CAg-3'	**85 (341)** 5'-CAg-3'	**67 (286)** 5'-gAT-3'	**78 (321)** 5'-CAA-3'	**67 (286)** 5'-gAA-3'	**85 (341)** 5'-CAA-3'	**58 (260)** 5'-CCT-3'
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
DRB1 allele[4]												
***12:01:01**	**1**	**2**		**4**	**5**	**6**	**7**	**8**	**9**			
***12:01:02-12:01:03**	**1**	**2**		**4**	**5**	**6**	**7**	**8**				
***12:02:01, 12:02:03-12:02:05**	**1**	**2**		**4**	**5**	**6**	**7**		**9**	**10**		
***12:02:02**	**1**	**2**		**4**	**5**	**6**	**7**			**10**		
***12:03:02**	**1**	**2**		**4**	**5**			**8**			**11**	
***12:04**	**1**	**2**		**4**		**6**	**7**	**8**	**9**			**12**
***12:05**	**1**	**2**			**5**	**6**	**7**	**8**				
***12:06**	**1**	**2**		**4**	**5**	**6**	**7**	**8**	**9**			
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

CE
0088

INTERPRETATION TABLE

DRB1*12 SSP subtyping

Amplification patterns of the DRB1*12:01 to 12:27 alleles

Well[5]												
13	14	15	16	17	18	19	20	21	22	23	24	
115	135	200	75	90	90	195	220	105	105	110	170	Length of spec.
185					135				220			PCR product(s)
255												
515	515	515	430	430	515	430	430	430	430	430	430	Length of int.
												pos. control[1]
13 (125)	149 (534)	16 (133)	25 (160)	-16 (40)	16 (133)	16 (133)	25 (160)	16 (133)	26 (165)	65 (280)	16 (133)	5'-primer[2]
5' -gTg 3'	5' -CAg 3'	5' -gTT 3'	5' -TgT 3'	5' -CAA 3'	5' -gTT 3'	5' -gTT 3'	5' -TgT 3'	5' -gTT 3'	5' -TTA 3'	5' -AgC 3'	5' -gTT 3'	
37 (197)			26 (165)	65 (280)			25 (161)			152 (543)		
5' -gTT 3'			5' -TTC 3'	5' -AgC 3'			5' -gCT 3'			5' -gAT 3'		
61 (269)												
5' -CTA 3'												
85 (341)	181 (630)	69 (293)	38 (199)	-3 (79)	32 (182)	67 (286)	85 (341)	38 (199)	47 (227)	85 (341)	59 (262)	3'-primer[3]
5' -CAg 3'	5' -CTT 3'	5' -CTC 3'	5' -CAg 3'	5' -AgC 3'	5' -TAC 3'	5' -gAg 3'	5' -CAg 3'	5' -CAg 3'	5' -ggT 3'	5' -CAg 3'	5' -CTg 3'	
				85 (341)	47 (226)				86 (344)	179 (624)		
				5' -CAg 3'	5' -gAg 3'				5' -CAC 3'	5' -ACA 3'		
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												DRB1 allele[4]
								21				*12:01:01
								21				*12:01:02-12:01:03
								21				*12:02:01, 12:02:03-12:02:05
								21				*12:02:02
								21				*12:03:02
								21				*12:04
13								21				*12:05
	14							21				*12:06
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

Length of spec.	**135**	**215**	**165**	**105**	**165**	**250**	**215**	**195**	**165**	**195**	**250**	**170**
PCR product(s)												
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
***12:07**	**1**	**2**		**4**	**5**	**6**	**7**		**9**			
***12:08**	**1**	**2**		**4**	**5**	**6**		**8**	**9**			
***12:09**	**1**	**2**	**3**	**4**		**6**	**7**	**8**	**9**			
***12:10**	**1**	**2**		**4**	**5**	**6**	**7**	**8**	**9**			
***12:11**	**1**	**2**		**4**	**5**	**6**	**7**	**8**	**9**			
***12:12**	**1**	**2**		**4**	**5**	**6**	**7**		**9**			
***12:13**	**1**	**2**		**4**	**5**	**6**	**w**		**9**	**10**		
***12:14**	**1**	**2**			**5**	**6**	**7**	**8**				
***12:15**	**1**	**2**			**5**	**6**	**7**			**10**		
***12:16**	**1**	**2**		**4**	**5**					**10**		
***12:17**	**1**	**2**		**4**	**5**	**6**	**7**	**8**	**9**			
***12:18**	**1**	**2**		**4**		**6**	**7**		**9**	**10**		
***12:19**	**1**	**2**		**4**	**5**				**9**	**10**	**11**	
***12:20**	**1**	**2**		**4**	**5**	**6**	**7**		**9**	**10**		
***12:21**		**2**		**4**	**5**	**6**	**7**		**9**	**10**		
***12:22**	**1**			**4**	**5**			**8**				
***12:23**	**1**	**2**		**4**	**5**	**6**			**9**	**10**		
***12:24N**	**1**	**2**		**4**	**w**	**6**	**7**	**8**	**9**			
***12:25**	**1**	**2**		**4**	**5**	**6**	**7**	**8**	**9**			
***12:26**	**1**	**2**		**4**	**5**	**6**	**7**		**9**	**10**		
***12:27**	**1**	**2**		**4**	**5**	**6**			**9**	**10**		
***01:01:01-01:36**												
***08:01:01-08:01:05, 08:07-08:08, 08:11, 08:16, 08:26, 08:39, 08:43**										**10**		
***08:02:01-08:02:03, 08:09, 08:21, 08:42, 08:44**			**3**							**10**		
***08:03:02, 08:14-08:15, 08:23, 08:27, 08:29, 08:30:02, 08:33, 08:35-08:36, 08:38**								**8**				
***08:04:01, 08:04:04-08:04:05, 08:04:07, 14:15**			**3**							**10**	**11**	
***08:04:02-08:04:03**			**3**							**10**	**w**	
***08:04:06, 08:06, 14:73**										**10**	**11**	
***08:05**		**2**								**10**		
***08:10**								**8**			**11**	
***08:12**						**6**		**8**				
***08:13**			**3**									
***08:17**	**1**									**10**		
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

115	135	200	75	90	90	195	220	105	105	110	170	Length of spec.
185					135				220			PCR product(s)
255												
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
		15						21				*12:07
			16					21				*12:08
								21				*12:09
				17				21				*12:10
					18			21				*12:11
						19		21				*12:12
							20	21				*12:13
13												*12:14
13								21				*12:15
								21	22			*12:16
								21		23		*12:17
								21			24	*12:18
								21				*12:19
13								21				*12:20
								21	22			*12:21
								21	22			*12:22
			16				20	21				*12:23
13								21				*12:24N
				17				21		23		*12:25
					18			21				*12:26
			16					21				*12:27
										23		*01:01:01-01:36
												*08:01:01-08:01:05, 08:07-08:08, 08:11, 08:16, 08:26, 08:39, 08:43
												*08:02:01-08:02:03, 08:09, 08:21, 08:42, 08:44
												*08:03:02, 08:14-08:15, 08:23, 08:27, 08:29, 08:30:02, 08:33, 08:35-08:36, 08:38
												*08:04:01, 08:04:04-08:04:05, 08:04:07, 14:15
												*08:04:02-08:04:03
												*08:04:06, 08:06, 14:73
												*08:05
												*08:10
												*08:12
						19						*08:13
												*08:17
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

Length of spec. PCR product(s)	**135**	**215**	**165**	**105**	**165**	**250**	**215**	**195**	**165**	**195**	**250**	**170**
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
***08:18, 08:40**		**2**						**8**				
***08:19, 08:34**					**5**			**8**				
***08:22**						**6**				**10**		
***08:24**		**2**	**3**							**10**		
***08:25**		**2**			**5**			**8**				
***08:28**	**1**		**3**							**10**	**11**	
***08:30:01**			**3**					**8**				
***08:31**		**2**								**10**	**11**	**12**
***08:32**				**4**				**8**				
***08:37**	**1**							**8**				
***08:41**		**2**								**10**		**12**
***08:45**	**1**		**3**					**8**				
***11:67**	**1**									**10**	**11**	**12**
***11:76, 13:34, 13:64, 14:41, 14:77, 14:110**												
***13:17**	**1**	**2**	**3**								**11**	
***13:116, 14:52**		**2**	**3**								**11**	
***14:04, 14:50, 14:76, 14:79, 14:107**											**11**	
***14:11**											**11**	**12**
***14:28**						**6**						
***14:31**		**2**									**11**	
***15:01:01:01-15:01:16, 15:02:01-15:57, 16:01:01-16:05:02, 16:07-16:18**												
DRB1 allele[4]												
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

[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 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.
Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB1*12 subtyping.
In addition, wells number 5, 13 to 15 and 18 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.
[2]The codon, and in parenthesis the nucleotide, in the 1st, 2nd or 3rd exon matching the specificity-determining 3'-end of the primer is given. Codon and 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 codon, and in parenthesis the nucleotide, in the 1st, 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Codon and 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.

115	135	200	75	90	90	195	220	105	105	110	170	Length of spec.
185					135				220			PCR product(s)
255												
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												*08:18, 08:40
												*08:19, 08:34
												*08:22
												*08:24
												*08:25
												*08:28
												*08:30:01
												*08:31
								21	22			*08:32
												*08:37
												*08:41
												*08:45
												*11:67
			16									*11:76, 13:34, 13:64, 14:41, 14:77, 14:110
												*13:17
												*13:116, 14:52
												*14:04, 14:50, 14:76, 14:79, 14:107
												*14:11
												*14:28
												*14:31
	w											*15:01:01:01-15:01:16, 15:02:01-15:57, 16:01:01-16:05:02, 16:07-16:18
												DRB1 allele[4]
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

[4]The sequence of the DRB1*12031 allele has been shown to be identical to DRB1*12:01:01.
[5]Primer mix 13: Specific PCR fragment of 115 bp in the DRB1*12:24N allele. Specific PCR fragment of 185 bp in the DRB1*12:05, 12:14 and 12:15 alleles. Specific PCR fragment of 255 bp in the DRB1*12:20 allele.
Primer mix 18: Specific PCR fragment of 90 bp in the DRB1*12:26 allele. Specific PCR fragment of 135 bp in the DRB1*12:11 allele.
Primer mix 22: Specific PCR fragment of 105 bp in the DRB1*12:21 allele. Specific PCR fragment of 220 bp in the DRB1*12:16 and the DRB1*12:22 alleles. Specific PCR fragment of 105 and 220 bp in the DRB1*08:32 allele.
'w', may be weakly amplified.

CELL LINE VALIDATION SHEET

DRB1*12 SSP subtyping kit

					Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.:	201071501	201071502	201071503	201071504	201071505	201071506	201071507	201071508	201071509	201071510	201071511	201071512	201076813	201071514	201071515	201076816
	IHWC cell line		DRB1																	
1	9001	SA	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*15:02	*04:05	-	-	-	-	-	-	-	-	-	-	-	-	-	W	-	-
3	9011	E4181324	*15:02		-	-	-	-	-	-	-	-	-	-	-	-	-	W	-	-
4	9275	GU373	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*16:01		-	-	-	-	-	-	-	-	-	-	-	-	-	W	-	-
6	9353	SM	*04:07	*08:03	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
7	9020	QBL	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025	DEU	*04:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*04:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*04:05		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*08:02		-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
15	9075	DKB	*09:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*03:01	*13:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*09:01	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*12:01		+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-
20	9059	SLE005	*13:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*14:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*13:02	*14:54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*08:03	*14:14	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
24	9035	JBUSH	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*04:05	*10:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*04:16	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*03:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*13:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*04:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*04:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*04:03	*04:06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*13:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*14:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*03:01	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*07:01	*09:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*13:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*08:03		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
43	9076	T7526	*09:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*14:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*15:01		-	-	-	-	-	-	-	-	-	-	-	-	-	W	-	-
47	9045	TUBO	*11:04	*12:01	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-
48	9303	TER-ND	*01:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CELL LINE VALIDATION SHEET

DRB1*12 SSP subtyping kit

					Well							
					17	18	19	20	21	22	23	24
				Prod. No.:	201191917	201076818	201071519	201191920	201071521	201076822	201076823	201071524
	IHWC cell line		DRB1									
1	9001	SA	*01:01		-	-	-	-	-	-	+	-
2	9280	LK707	*15:02	*04:05	-	-	-	-	-	-	-	-
3	9011	E4181324	*15:02		-	-	-	-	-	-	-	-
4	9275	GU373	*03:01		-	-	-	-	-	-	-	-
5	9009	KAS011	*16:01		-	-	-	-	-	-	-	-
6	9353	SM	*04:07	*08:03	-	-	-	-	-	-	-	-
7	9020	QBL	*03:01		-	-	-	-	-	-	-	-
8	9025	DEU	*04:01		-	-	-	-	-	-	-	-
9	9026	YAR	*04:02		-	-	-	-	-	-	-	-
10	9107	LKT3	*04:05		-	-	-	-	-	-	-	-
11	9051	PITOUT	*07:01		-	-	-	-	-	-	-	-
12	9052	DBB	*07:01		-	-	-	-	-	-	-	-
13	9004	JESTHOM	*01:01		-	-	-	-	-	-	+	-
14	9071	OLGA	*08:02		-	-	-	-	-	-	-	-
15	9075	DKB	*09:01		-	-	-	-	-	-	-	-
16	9037	SWEIG007	*11:01		-	-	-	-	-	-	-	-
17	9282	CTM3953540	*03:01	*13:01	-	-	-	-	-	-	-	-
18	9257	32367	*09:01	*11:01	-	-	-	-	-	-	-	-
19	9038	BM16	*12:01		-	-	-	-	+	-	-	-
20	9059	SLE005	*13:02		-	-	-	-	-	-	-	-
21	9064	AMALA	*14:02		-	-	-	-	-	-	-	-
22	9056	KOSE	*13:02	*14:54	-	-	-	-	-	-	-	-
23	9124	IHL	*08:03	*14:14	-	-	-	-	-	-	-	-
24	9035	JBUSH	*11:01		-	-	-	-	-	-	-	-
25	9049	IBW9	*07:01		-	-	-	-	-	-	-	-
26	9285	WT49	*03:01		-	-	-	-	-	-	-	-
27	9191	CH1007	*04:05	*10:01	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*04:16	*07:01	-	-	-	-	-	-	-	-
29	9050	MOU	*07:01		-	-	-	-	-	-	-	-
30	9021	RSH	*03:02		-	-	-	-	-	-	-	-
31	9019	DUCAF	*03:01		-	-	-	-	-	-	-	-
32	9297	HAG	*13:03		-	-	-	-	-	-	-	-
33	9098	MT14B	*04:04		-	-	-	-	-	-	-	-
34	9104	DHIF	*11:01		-	-	-	-	-	-	-	-
35	9302	SSTO	*04:03		-	-	-	-	-	-	-	-
36	9024	KT17	*04:03	*04:06	-	-	-	-	-	-	-	-
37	9065	HHKB	*13:01		-	-	-	-	-	-	-	-
38	9099	LZL	*14:02		-	-	-	-	-	-	-	-
39	9315	CML	*03:01	*04:01	-	-	-	-	-	-	-	-
40	9134	WHONP199	*07:01	*09:01	-	-	-	-	-	-	-	-
41	9055	H0301	*13:02		-	-	-	-	-	-	-	-
42	9066	TAB089	*08:03		-	-	-	-	-	-	-	-
43	9076	T7526	*09:01		-	-	-	-	-	-	-	-
44	9057	TEM	*14:01		-	-	-	-	-	-	-	-
45	9239	SHJO	*07:01		-	-	-	-	-	-	-	-
46	9013	SCHU	*15:01		-	-	-	-	-	-	-	-
47	9045	TUBO	*11:04	*12:01	-	-	-	-	+	-	-	-
48	9303	TER-ND	*01:03		-	-	-		-	-	+	-

CERTIFICATE OF ANALYSIS

Olerup SSP® DRB1*12 SSP

Product number: **101.128-12u – without *Taq* polymerase**
Lot number: **83M**
Expiry date: **2014-April-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	2010-715-01	9	2010-715-09	17	2011-919-17
2	2010-715-02	10	2010-715-10	18	2010-768-18
3	2010-715-03	11	2010-715-11	19	2010-715-19
4	2010-715-04	12	2010-715-12	20	2011-919-20
5	2010-715-05	13	2010-768-13	21	2010-715-21
6	2010-715-06	14	2010-715-14	22	2010-768-22
7	2010-715-07	15	2010-715-15	23	2010-768-23
8	2010-715-08	16	2010-768-16	24	2010-715-24

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 13, 15, 16, 18 to 20, 22 and 24 were available. The specificities of the primers in primer solutions 16, 19, 20 and 22 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 15, 18 and 24 it was only possible to test the 5'-primer, the 3'-primers were not possible to test. In primer solutions 13 it was only possible to test the 3'-primer, the 5'-primers were not possible to test. In primer solutions 17 and 23 one 5'-primer was not possible to test. One additional 3'-primer in primer solutions 17 and 23 was tested by separately adding one additional 5'-primer.

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

Date of approval: 2011-October-26

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*12
Product number: 101.128-12u
Lot number: 83M

Intended use: DRB1*12 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Franzengatan 5
SE-112 51 Stockholm, 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 9001:2008 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, Franzengatan 5, SE-112 51 Stockholm, Sweden.

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

Stockholm, Sweden
2011-October-26

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

ADDRESSES:

Manufacturer:
***Olerup* SSP AB**, Franzengatan 5, SE-112 51 Stockholm, Sweden.
Tel: +46-8-717 88 27
Fax: +46-8-717 88 18
E-mail: info-ssp@olerup.com
Web page: http://www.olerup-ssp.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**.