

101.103.48/12 – including *Taq* pol., IFU-01
 101.103.48u/12u – without *Taq* pol., IFU-02

Visit www.olerup-ssp.com for
 “Instructions for Use” (IFU)

Lot No.: **38Y**

Lot-specific information

Olerup SSP® DR low resolution screening

Product number:	101.103-48/12 – including <i>Taq</i> pol. 101.103-48u/12u – without <i>Taq</i> pol.
Lot number:	38Y
Expiry date:	2017-October-01
Number of tests:	48 tests – Product No. 101.103-48/48u 12 tests – Product No. 101.103-12/12u
Number of wells per test:	23 + 1
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. 38Y.

Complete product documentation consists of generic Instructions for Use (IFU), lot specific Product Insert, Worksheet and Certificate.

**CHANGES COMPARED TO THE PREVIOUS OLERUP SSP®
 DR LOW RESOLUTION SCREENING LOT (42V)**

The format of the Product Insert and Worksheet have been changed.

The **DR low resolution Screening** specificity and interpretation tables have been updated for the HLA-DRB alleles described since the previous *Olerup SSP®* DR low resolution Screening lot was made (**Lot No. 42V**). The kit design is based on IMGT/HLA database 3.18.0.

As of lot series V, the Specificity Table is included in the lot-specific Product Insert, and the Interpretation Table is included in the Worksheet.

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

Well	5'-primer	3'-primer	rationale
3	Added	Added	5'-primer added for the DRB1*15:01:22 allele, 3'-primer added for the DRB1*15:115N allele.
4	-	Exchanged	3'-primer exchanged for improved HLA-specific amplification.
5	-	Added	3'-primer added for the DRB1*03:95 allele.
6	-	Added	3'-primer added for the DRB1*13:193 allele.
10	Added	-	5'-primer added for the DRB1*08:01:06 allele.
11	Added	-	5'-primer added for the DRB1*09:23 allele.
24	-	-	Updated negative control.

Change in revision R01 compared to R00:

1. Primer mix 5 does not amplify the DRB1*03:11:01, *03:97, *11:53 and *15:100 alleles. This has been corrected in the Specificity and Interpretation Tables.

Changes in revision R02 compared to R01:

1. Due to sharing of sequence motifs, DRB3*01:23 will be amplified in primer mix 5, in addition to primer mix 21.

2. The DRB1*13:02:02 allele is amplified in primer mix 6. Thus, the DRB1*03:11:02 and DRB1*13:02:02 alleles will give rise to identical amplification patterns with the DR low resolution screening primer set. These alleles can be separated by the respective high resolution primer sets. The Specificity and Interpretation Tables have been changed.

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Well **24** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup SSP*® HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

HLA-specific PCR product sizes range from 75 to 200 base pairs.
The PCR product generated by the positive control primer pair is 430 base pairs.

Length of PCR product	105	200	105	80	75	80	85
5'-primer¹	164	340	440	45	45	43	36
	5'-CAC ^{3'}	5'-Agg ^{3'}	5'-TTA ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-TAC ^{3'}
							36
							5'-TAT ^{3'}
3'-primer²	231	2nd I	507	59	58	57	47
	5'-TgC ^{3'}	5'-AAA ^{3'}	5'-TTg ^{3'}	5'-CTC ^{3'}	5'-ggC ^{3'}	5'-CTC ^{3'}	5'-ACA ^{3'}
							48
							5'-gCA ^{3'}
							48
							5'-gCC ^{3'}
							52
							5'-TgT ^{3'}
A*	+	+	+				
B*	+	+	+				
C*	+	+	+				
DRB1				+	+		
DRB3				+	+		
DRB5				+			
DQB1					+		
DPB1						+	
DQA1							+

¹The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

²The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

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Lot-specific information

PRODUCT DESCRIPTION

DR low resolution screening

CONTENT

The primer set contains 5'- and 3'-primers for grouping the DRB1*01:01 to DRB1*10:07 alleles into the corresponding serological groups DR1 to DR18 as well as primer pairs for recognizing the DRB3, DRB4 and DRB5 groups of 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	NC

The 24 well cut PCR plate is marked with ‘DR low screening’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘38Y’.

Wells 1 to 23 – DR low resolution screening primers.

Well 24 – Negative Control.

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

Only HLA-DRB alleles will be amplified by the 23 wells of the DR low resolution screening primer set, **wells 1 to 23**. Thus, the interpretation of DR low resolution screening typings is not influenced by other HLA class II genes.

UNIQUELY IDENTIFIED ALLELES

All the HLA-DRB1, -DRB3, -DRB4¹ and -DRB5 alleles, i.e. **DRB1*01:01 to 10:07, DRB3*01:01 to DRB3*03:03, DRB4*01:01:01:01 to DRB4*01:08, DRB5*01:01:01 to DRB5*02:06**, recognized by the HLA Nomenclature Committee in October 2014^{2,3} will be amplified by the primers in the DR low resolution screening SSP kit. The HLA-DRB alleles will be grouped into their corresponding serological specificities.

The following alleles give rise to identical amplification patterns with the DR low resolution screening primer set. These alleles can be separated by the respective high resolution primer sets.

Alleles	Alleles
DRB1*03:76, 13:176	DRB1*08:31, 08:41, 11:67
DRB1*03:11:02, 13:02:02	DRB1*13:13, 13:119, 13:154, 13:156, 14:84, 14:116, 14:144
DRB1*08:20, 13:18, 13:47, 13:55, 13:158, 13:164	

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¹The DRB1*13:183, DRB4*02:01N and DRB4*03:01N null alleles will not be amplified by the DR low resolution screening primer set.

²DRB alleles listed on the IMGT/HLA web page 2014-October-10, release 3.18.0, www.ebi.ac.uk/imgt/hla.

³Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page <http://hla.alleles.org/alleles/deleted.html>.

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Lot-specific information
SPECIFICITY TABLE

DR low resolution screening primer set

Specificities and sizes of the PCR products of the 23+1 primer mixes of the DR low resolution screening primer set

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	DR serology ³	Amplified HLA-DRB ⁴ alleles
1^{6,7}	210 bp, 235 bp, 260 bp	515 bp	1	*01:01:01-01:02:10, 01:04-01:38, 01:40N-01:65
2	200 bp	430 bp	1/103	*01:03, 01:39N, 01:42, 01:61
3^{6,7,9}	210 bp, 230 bp	430 bp	2, 15	*15:01:01:01-15:115N
4⁹	210 bp	430 bp	16	*16:01:01-16:05:02, 16:07-16:24
5^{5,6,12}	120 bp, 220 bp	430 bp	3, 11, 17, 18	*03:01:01:01-03:10, 03:12-03:75, 03:77-03:96, 03:98-03:115, 11:07, 11:103, 11:105, 11:107, 11:125, 15:25
6^{5,6,8,12}	75 bp, 210 bp	430 bp	3, 6, 11, 13, 14, 17	*03:01:01:01-03:01:23, 03:04:01-03:06, 03:08-03:16, 03:18-03:20, 03:22-03:23, 03:25-03:26, 03:28, 03:30-03:31, 03:33-03:34, 03:36-03:37, 03:43-03:48, 03:50-03:52, 03:54-03:68N, 03:70-03:73, 03:75-03:86, 03:89, 03:91-03:93, 03:95-03:96, 03:98-03:100:02, 03:104, 03:106-03:110, 03:112-03:114, 08:40, 11:02:01-11:03, 11:11:01-11:11:03, 11:14:01-11:14:02, 11:16, 11:20-11:21, 11:36, 11:40-11:41, 11:48, 11:59, 11:63:01-11:63:02, 11:65:01-11:65:02, 11:68, 11:70, 11:73, 11:76, 11:79-11:80, 11:83, 11:85-11:87, 11:93, 11:118, 11:122, 11:124, 11:127, 11:131-11:132, 11:135, 11:138-11:139, 11:142, 11:151, 11:153, 11:161, 11:168, 11:171, 13:01:01-13:04, 13:08, 13:10, 13:15-13:17, 13:19-13:20, 13:22-13:24, 13:27-13:29, 13:31-13:41, 13:43, 13:45, 13:48, 13:51-13:54, 13:57, 13:59, 13:61:01-13:61:02, 13:63-13:66:02, 13:68-13:76, 13:78-13:81, 13:83-13:85, 13:87-13:99, 13:101-13:102, 13:104-13:107, 13:109, 13:111-13:117, 13:120-13:131, 13:133, 13:135, 13:137N-13:145, 13:147-13:149, 13:151-13:153, 13:155, 13:159, 13:162, 13:165-13:168, 13:170-13:180, 13:182, 13:184-13:188, 13:190-13:191, 13:193, 14:16, 14:19, 14:21, 14:45, 14:82, 14:95, 14:109, 14:120, 14:122, 14:132, 14:137N, 14:152N
7^{5,6}	85 bp, 210 bp	430 bp	3, 6, 11, 13, 14, 1403, 18	*03:02:01-03:03, 03:27, 03:29, 03:38, 03:53, 03:74, 03:88, 03:90, 03:102-03:103, 03:115, 11:13:01 ^w -11:13:02 ^w , 11:26, 11:34, 13:15, 13:19, 13:26:01-13:26:02, 13:44, 13:53, 13:57, 13:85-13:86, 13:104, 13:193, 14:02:01-

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				14:03:02, 14:06:01-14:06:03, 14:09, 14:12:01-14:13, 14:17-14:21, 14:24, 14:27, 14:29-14:30, 14:32:01 ^w -14:32:02 ^w , 14:33, 14:40-14:41, 14:47-14:49, 14:51, 14:63, 14:65 ^w , 14:67, 14:77-14:78, 14:80-14:81, 14:83, 14:85, 14:89, 14:94, 14:98, 14:102, 14:106, 14:108-14:109, 14:115, 14:119, 14:121, 14:135, 14:146, 14:154, 14:156, 14:159
8^{5,6,7}	100 bp, 175 bp	430 bp	3, 4	*04:01:01-04:05:11, 04:05:13-04:186N
9^{6,7,9}	200 bp, 235 bp	430 bp	7, 13, 14	*07:01:01:01-07:01:08, 07:03-07:35, 12:22, 13:17, 13:116, 13:175, 14:50
10⁶	170 bp, 215 bp, 250 bp	515 bp	8, 11, 12, 14	*08:01:01-08:19, 08:21-08:56, 08:58-08:60N, 11:67, 12:04, 12:16:01-12:16:03, 12:22, 12:39, 12:49, 14:11, 14:15, 14:68:01, 14:93, 14:148
11^{5,6}	90 bp, 135 bp, 165 bp, 190 bp	430 bp	3, 9, 11	*03:08, 03:65, 03:112, 09:01:02-09:24, 11:07, 11:103, 11:105, 11:107, 11:125
12	180 bp	430 bp	10	*03:76, 10:01:01-10:07, 11:59, 11:80, 11:83, 11:87, 11:135, 11:142, 13:27, 13:41, 13:71, 13:129, 13:176
13^{5,6}	100 bp, 170 bp	430 bp	3, 8, 11, 13, 14	*03:08, 03:65, 08:31, 08:41, 11:01:01-11:70, 11:72-11:171
14^{5,6}	90 bp, 110 bp	430 bp	12	*08:32, 08:53, 12:01:01-12:51
15^{7,13}	220 bp	430 bp	6, 8, 11, 13, 14, 1403	*03:76, 08:20-08:21, 11:01:01-11:04:11, 11:06:01-11:06:03, 11:08:01-11:12:02, 11:14:01-11:16, 11:18-11:21, 11:23:01-11:25, 11:27:01-11:33, 11:35-11:51, 11:54:01-11:54:02, 11:56-11:66, 11:68, 11:70, 11:72-11:81, 11:83-11:88, 11:90-11:97, 11:99-11:102:02, 11:106, 11:108-11:124, 11:126-11:135, 11:137-11:142, 11:144-11:158, 11:160-11:171, 13:01:01-13:02:01, 13:02:03-13:08, 13:10-13:16, 13:18-13:43, 13:45-13:85, 13:87-13:115, 13:117-13:128, 13:130-13:145, 13:147-13:166, 13:168-13:174, 13:176-13:182, 13:184-13:191, 14:03:01-14:03:02, 14:12:01-14:12:02, 14:16, 14:19, 14:21-14:22, 14:25, 14:27, 14:40, 14:53, 14:63, 14:67, 14:69, 14:74, 14:77-14:78, 14:84-14:85, 14:98, 14:102, 14:105, 14:109, 14:115-14:116, 14:128, 14:135, 14:137N, 14:144, 14:156, DRB3*02:27
16^{6,7}	200 bp, 225 bp	430 bp	6, 8, 11, 12, 13, 14	*08:01:01-08:01:05, 08:02:01-08:02:04, 08:04:01-08:09, 08:11, 08:16-08:17, 08:20-08:22, 08:24, 08:26, 08:28, 08:31, 08:39, 08:41-08:44, 08:50, 08:52, 08:54-08:55, 08:57, 08:59, 11:01:01-11:01:17, 11:01:20-11:06:03, 11:09-11:12:02, 11:14:01-11:16, 11:20-11:21, 11:23:01-11:25, 11:27:01-11:30, 11:32-11:33, 11:35-11:41, 11:43-11:44, 11:46:01-11:51, 11:54:01-11:56, 11:58:01-11:63:02, 11:65:01-

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17 ^{6,12}	175 bp, 240 bp	430 bp	3, 6, 8, 11, 13, 14, 17, 18	*03:01:01:01-03:01:05, 03:01:07-03:01:08, 03:01:10-03:07, 03:09, 03:11:01-03:41, 03:43-03:45, 03:47-03:63, 03:66-03:68N, 03:70-03:86, 03:88-03:91, 03:93-03:110, 03:112-03:115, 08:20, 11:13:01-11:13:02, 11:149, 13:01:01-13:16, 13:18-13:42, 13:44, 13:46-13:66:02, 13:68-13:102, 13:104-13:115, 13:117-13:121, 13:123-13:158, 13:161-13:164, 13:166-13:170, 13:172-13:174, 13:176-13:178, 13:180-13:182, 13:184-13:190, 13:192-13:193, 14:01:01-14:14, 14:15 [?] -14:16 [?] , 14:17-14:21, 14:22 [?] , 14:23:01, 14:23:02 [?] , 14:23:03-14:24, 14:25 [?] -14:26 [?] , 14:27, 14:28 [?] , 14:29-14:30, 14:31 [?] -14:32:03 [?] , 14:33, 14:34 [?] -14:35 [?] , 14:36-14:37, 14:38:01 [?] -14:39 [?] , 14:40-14:45, 14:47-14:48, 14:49 [?] -14:50 [?] , 14:51, 14:52 [?] -14:53 [?] , 14:54:01-14:54:05, 14:55 [?] , 14:56-14:57, 14:58 [?] , 14:59, 14:60 [?] -14:62 [?] , 14:63-14:65, 14:67, 14:68:01 [?] -14:76 [?] , 14:77-14:78, 14:79 [?] , 14:80-14:85, 14:86 [?] -14:88 [?] , 14:89, 14:90 [?] , 14:91, 14:92N [?] -14:93 [?] , 14:94-14:96, 14:97 [?] , 14:98, 14:99 [?] , 14:100, 14:101 [?] , 14:102-14:103, 14:104 [?] -14:105 [?] , 14:106, 14:107 [?] , 14:108-14:109, 14:110 [?] -14:114 [?] , 14:115-14:116, 14:117 [?] -14:120 [?] , 14:121, 14:122 [?] , 14:123, 14:124 [?] -14:126:01 [?] , 14:127:01-14:127:02, 14:128 [?] -14:133 [?] , 14:134-14:137N, 14:138 [?] -14:140 [?] , 14:141, 14:142 [?] -14:143 [?] , 14:144, 14:145 [?] -14:151 [?] , 14:152N, 14:153 [?] , 14:154-14:156, 14:157 [?] -14:158 [?] , 14:159

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18 ^{5,6,11}	100 bp, 150 bp, 195 bp, 240 bp	430 bp	4, 6, 8,13, 14, 1404	*04:62, 04:69, 04:73, 04:105:01-04:105:02, 04:122, 04:146, 08:08, 11:69, 11:82, 13:45, 14:01:01-14:01:02, 14:01:04, 14:04:01, 14:07:01-14:07:02, 14:10, 14:16, 14:22, 14:25- 14:26, 14:28, 14:31-14:32:03, 14:35, 14:37- 14:39, 14:49-14:50, 14:53-14:54:01, 14:54:03- 14:55, 14:57-14:58, 14:60-14:62, 14:68:01- 14:71, 14:73-14:76, 14:79, 14:82, 14:86-14:88, 14:90, 14:93, 14:99, 14:101, 14:104-14:105, 14:107, 14:110-14:114, 14:117-14:120, 14:122, 14:124-14:125, 14:128-14:129, 14:131, 14:137N-14:140, 14:142-14:143, 14:145-14:147, 14:149-14:153, 14:157-14:158, DRB4*01:03:01:02N
19 ^{5,6,10}	110 bp, 145 bp, 170 bp	430 bp	3, 4, 6, 9, 11, 13, 14, 1404	*03:10, 09:01:02-09:01:05, 09:01:07-09:02:02, 09:04-09:22, 09:24, 11:13:01-11:13:02, 11:17, 11:52, 13:43, 13:159, 13:171:01, 13:179, 13:191, 13:193, 14:01:01-14:02:02, 14:04:01- 14:11, 14:13-14:14, 14:16-14:18, 14:19 ^w , 14:20, 14:21 ^w , 14:22-14:23:04, 14:26, 14:28- 14:36, 14:38:01-14:39, 14:41, 14:43-14:52, 14:54:01-14:54:03, 14:54:04 ^w , 14:54:05-14:57, 14:59-14:62, 14:64-14:65, 14:68:01, 14:70- 14:76, 14:79-14:83, 14:86-14:88, 14:90-14:97, 14:99-14:101, 14:103-14:108, 14:109 ^w , 14:110-14:114, 14:117-14:127:02, 14:129- 14:134, 14:137N-14:140, 14:142-14:143, 14:145-14:155, 14:157-14:159, 15:27, 15:34, 15:66:01-15:66:02
20 ^{5,6,7}	110 bp, 150 bp, 180 bp, 220 bp	430 bp	2 ^w , 3, 4, 6, 8, 11, 13, 14, 1403, 1404, 16 ^w	*03:10, 08:09, 08:20-08:21, 08:32, 08:35, 08:36:02, 08:53, 11:13:01-11:13:02, 11:17, 11:23:01-11:23:02, 11:25, 11:31, 11:45, 11:52, 11:55, 11:64, 11:89, 11:96, 11:119, 11:148, 11:159, 13:13, 13:18, 13:43, 13:45, 13:47, 13:55, 13:119, 13:144, 13:146, 13:154, 13:156, 13:158-13:159, 13:164, 13:171:01, 13:179, 13:191, 14:01:01-14:01:04, 14:03:01-14:05:04, 14:07:01-14:08, 14:10-14:12:02, 14:14-14:16, 14:18, 14:22-14:23:04, 14:25-14:28, 14:31- 14:32:03, 14:34-14:36, 14:38:01-14:40, 14:42- 14:45, 14:49-14:50, 14:53-14:65, 14:67-14:79, 14:81-14:82, 14:84-14:93, 14:95-14:97, 14:99- 14:105, 14:107, 14:110-14:120, 14:122- 14:140, 14:142-14:158, 15:21 ^w , 16:04 ^w , 16:18 ^w
21 ^{6,12,13}	160 bp, 240 bp	430 bp	52	*14:141, DRB3*01:01:02:01-01:15, DRB3*02:01:02:29N, DRB3*03:01:01-03:03
22 ^{5,7,11}	215 bp	430 bp	53	DRB4*01:01:01-01-01:08
23	175 bp	430 bp	51	DRB5*01:01:01-01:15, DRB5*02:02-02:06
24 ¹⁴	-	-		Negative control

¹Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of DR low resolution screening SSP subtypings. When the primers in a primer mix can give rise to HLA-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits, the alleles listed are specified according to amplicon length.

101.103.48/12 – including Taq pol., IFU-01
101.103.48u/12u – without Taq pol., IFU-02

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Lot No.: 38Y

Lot-specific information

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, e.g. the primers in wells 3, 18, 19 and 20.

PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the longer, 515 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

³The serological reactivity of all DRB alleles is not known. In this table we use the information in the HLA Dictionary 2004 on the www.ebi.ac.uk/imgt/hla web site and the expert-assigned serological grouping in Tissue Antigens (2009) 73:95-170.

⁴For several DRB alleles 1st and/or 3rd exon(s) and beyond, as well as intron nucleotide sequences, are not 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. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

⁵HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁶Individual alleles can give rise to two differently sized specific PCR fragments in primer mix 1, 3, 5 to 11, 13, 14, 16 to 21.

⁷Primer mixes 1, 3, 8, 9, 15, 16, 20 and 22 have a tendency to giving rise to primer oligomer formation.

⁸Primer mix 6 may give a lower yield of HLA-specific PCR product than the other DR low primer mixes.

⁹Primer mixes 3, 4 and 9 may have tendencies of unspecific amplification.

¹⁰Primer mix 19 has a tendency of primer oligomer formation and also has an intense primer cloud due to the high number of primers present in the primer mix.

¹¹The DRB4*01:03:01:02N allele is amplified by the primer pairs in wells 18 and 22, whereas the DRB4*02:01N and DRB4*03:01N null alleles are not amplified by these primer pairs.

¹²Due to sharing of sequence motifs in codon 38 and 47, DRB3*01:14 will also be amplified in primer mixes 5, 6 and 17, and DRB3*01:23 in mix 5, in addition to primer mix 21.

¹³Due to sharing of sequence motifs, DRB3*02:27 is amplified by the primer pairs in well 15 in addition to primer mix 21.

¹⁴Primer mix 24 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by the control primer pairs matching the human growth hormone gene. HLA-specific PCR product sizes range from 75 to 200 base pairs and the PCR product generated by the HGH positive control primer pair is 430 base pairs.

‘w’, might be weakly amplified.

‘?’ , nucleotide sequence of the primer matching region not known.

101.103.48/12 – including *Taq* pol., IFU-01
 101.103.48u/12u – without *Taq* pol., IFU-02

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Lot No.: **38Y**

Lot-specific information

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	210 235 260	200	210 230	210	120 220	75 210	85 210	100 175	200 230	170 215 250	90 135 165 190	180
Length of int. pos. control ¹	515	430	430	430	430	430	430	430	430	515	430	430
5'-primer(s) ²	12(124) 5'-A.T 3'	14(129) 5'-gAA 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	13(125) 5'-gTC 3'	13(125) 5'-gTC 3'	13(125) 5'-gTC 3'	13(125) 5'-ACA 3'	13(127) 5'-ATA 3'	15(133) 5'-gTT 3'	26(165) 5'-TAT 3'	26(164) 5'-gTA 3'
		14(129) 5'-gAA 3'	13(126) 5'-AAG 3'	13(126) 5'-AAG 3'	47(227) 5'-gTT 3'	15(133) 5'-gTT 3'		13(125) 5'-ACC 3'	13(127) 5'-ATA 3'	15(133) 5'-gTT 3'	36(196) 5'-AgA 3'	30(178) 5'-gCg 3'
			13(126) 5'-AgA 3'					13(125) 5'-ATA 3'	13(127) 5'-gTA 3'	15(133) 5'-gCT 3'	58(261) 5'-gAg 3'	
								13(125) 5'-gTC 3'	15(133) 5'-gTT 3'			
3'-primer(s) ³	66(286) 5'-gAg 3'	66(286) 5'-gAT 3'	66(286) 5'-gAT 3'	66(286) 5'-gAA 3'	73(305) 5'-ggC 3'	26(164) 5'-ggT 3'	28(171) 5'-CTC 3'	32(184) 5'-gTg 3'	70(298) 5'-CTC 3'	58(260) 5'-CCT 3'	57(257) 5'-CgA 3'	73(307) 5'-CgC 3'
	66(286) 5'-gAg 3'		66(286) 5'-gAT 3'	66(286) 5'-gAg 3'	73(305) 5'-ggC 3'	66(286) 5'-gAT 3'	69(295) 5'-CTg 3'	58(260) 5'-Cgg 3'	73(305) 5'-ggC 3'	73(307) 5'-CAg 3'	73(305) 5'-ggC 3'	
	66(286) 5'-gAT 3'		69(295) 5'-CTg 3'	70(296) 5'-TgT 3'	73(305) 5'-ggC 3'	71(299) 5'-gCT 3'			77(317) 5'-AAT 3'	86(344) 5'-CAC 3'	77(319) 5'-CAC 3'	
	70(297) 5'-CTg 3'		69(295) 5'-Tg 3'	71(301) 5'-ggC 3'	74(308) 5'-CCC 3'				77(319) 5'-CAC 3'			
	71(299) 5'-gCg 3'		70(298) 5'-CgC 3'		74(310) 5'-CAA 3'				77(319) 5'-CAA 3'			
	77(317) 5'-AgT 3'		71(299) 5'-gCT 3'									
	86(344) 5'-CCA 3'		73(305) 5'-ggC 3'									
			77(317) 5'-AgT 3'									
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15	16	17	18	19	20	21	22	23
Length of spec. PCR product	100 170	90 110	220	200 225	175 240	100 150 195 240	110 145 170	110 150 180 220	160 240	215	175
Length of int. pos. control ¹	430	430	430	430	430	430	430	430	430	430	430
5'-primer(s) ²	13(125) 5'-gTC 3'	12(124) 5'-Cgg 3'	10(116) 5'-gCT 3'	10(116) 5'-gCT 3'	13(125) 5'-gTC 3'	1st I 5'-CAA 3'	26(164) 5'-gTA 3'	13(125) 5'-gTC 3'	10(116) 5'-gCT 3'	28(170) 5'-gAT 3'	13(125) 5'-gTA 3'
	15(133) 5'-gTC 3'	15(133) 5'-gTT 3'	12(122) 5'-TAT 3'	12(122) 5'-TAT 3'	114(429) 5'-CTg 3'	37(197) 5'-gTT 3'	34(189) 5'-CAg 3'	34(189) 5'-CAg 3'	10(116) 5'-gCT 3'		
	38(200) 5'-CgT 3'		13(125) 5'-gTC 3'	13(125) 5'-gTC 3'		37(197) 5'-gTA 3'		36(196) 5'-AgC 3'	37(199) 5'-TCC 3'		
			15(133) 5'-gTT 3'								
			15(133) 5'-gTC 3'								
3'-primer(s) ³	58(260) 5'-CCT 3'	29(175) 5'-gTg 3'	69(295) 5'-gTC 3'	66(286) 5'-gAA 3'	58(260) 5'-Cgg 3'	42(213) 5'-TCA 3'	57(257) 5'-CAg 3'	57(257) 5'-CAg 3'	51(239) 5'-CCC 3'	86(346) 5'-CTC 3'	57(258) 5'-gCg 3'
	58(260) 5'-CCT 3'	37(199) 5'-CAg 3'	71(299) 5'-gCT 3'	70(298) 5'-CgC 3'	58(260) 5'-CAg 3'	57(257) 5'-CAg 3'	69(295) 5'-CTg 3'	59(265) 5'-gTg 3'	77(317) 5'-AAT 3'	86(346) 5'-CTT 3'	58(260) 5'-CCT 3'
	58(260) 5'-CCT 3'		71(299) 5'-ACT 3'	70(298) 5'-CTC 3'	181(630) 5'-CTT 3'	70(298) 5'-CgC 3'	70(296) 5'-TCC 3'	70(296) 5'-TCC 3'			
								73(307) 5'-CAg 3'			
Well No.	13	14	15	16	17	18	19	20	21	22	23

101.103.48/12 – including *Taq* pol., IFU-01
101.103.48u/12u – without *Taq* pol., IFU-02

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Lot No.: **38Y**

Lot-specific information

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the longer, 515 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

²The nucleotide position matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

³The nucleotide position 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.

101.103.48/12 – including *Taq* pol., IFU-01
101.103.48u/12u – without *Taq* pol., IFU-02

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Lot No.: **38Y**

Lot-specific information

CELL LINE VALIDATION SHEET																				
DR low resolution primer set ³																				
				Well ²																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Prod. No.:	201440101	201440102	201551903	201550204	201439405	201550206	201440107	201440108	201445709	201439410	201550211	201440112	201440113	201440114	201440115	201550216
IHC cell line ¹		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		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

101.103.48/12 – including *Taq* pol., IFU-01
101.103.48u/12u – without *Taq* pol., IFU-02

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Lot No.: **38Y**

Lot-specific information

CELL LINE VALIDATION SHEET										
DR low resolution primer set ³										
			Well ²							
			17	18	19	20	21	22	23	
			Prod. No.:	201551917	201440118	201550219	201550220	201440129	201440130	201440131
	IHWC cell line ¹	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		-	-	-	-	-	-	-

¹The provided cell line HLA specificities are retrieved from the <http://www.ihwg.org/hla> web site. The specificity of an individual cell line may thus be subject to change.

101.103.48/12 – including *Taq* pol., IFU-01
101.103.48u/12u – without *Taq* pol., IFU-02

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²The DRB4*01:03:01:02N allele is amplified by primer mix 18 in the 9052 (DBB) cell line.

³The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs.

Additional 5'- and 3'-primers in primer solutions 9, 11 and 18 were tested by separately adding one 3'-primer, respectively one 5'-primer.

Additional 5'-primers in primer solutions 6, 12, 15 and 16 were tested by separately adding one 3'-primer. Additional 3'-primers in primer solutions 1, 3, 4, 10 and 20 were tested by separately adding one 5'-primer.

One, two or three of the 5'-primers in primer solution 1, 3, 4, 8, 9, 10, 13 to 16 and 20 were not possible to test. One or two of the 3'-primers in primer solution 1, 3 to 6, 9, 13, 15 and 22 were not possible to test.

101.103.48/12 – including *Taq* pol., IFU-01
101.103.48u/12u – without *Taq* pol., IFU-02

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Lot No.: **38Y**

Lot-specific information

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