

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

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“Instructions for Use” (IFU)

Lot No.: **2G1**

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:	2G1
Expiry date:	2020-10-01
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. 2G1.

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

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. 4E9**). The kit design is based on IMGT/HLA database 3.30.0.

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

Well	5'-primer	3'-primer	rationale
4	Removed, added	Added, removed	One 5'-primer and two 3'-primers removed and 3'-primers added for improved HLA-specific amplification, 5'-primer added for the DRB1*12:57 allele.
5	-	Added	3'-primer added for the DRB1*03:15:02 allele.
10	Removed, added	-	5'-primer removed for improved HLA-specific amplification, 5'-primer added for the DRB1*13:197 allele.
12	-	Added	3'-primer added for the DRB1*10:11 allele.
15	-	Added	3'-primer added for the DRB1*13:220 allele.
19	Added	-	5'-primers added for the DRB1*14:185 allele.

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Well **24** contains Negative Control primer pairs, that will amplify more than 95% of 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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PRODUCT DESCRIPTION

DR low resolution screening

CONTENT

The primer set contains 5'- and 3'-primers for grouping the DRB1*01:01 to DRB1*10:22 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. '2G1'.

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, -DRB4 and -DRB5 alleles, i.e. **DRB1*01:01:01 to DRB1*10:22**, **DRB3*01:01:02:01 to DRB3*03:13**, **DRB4*01:01:01:01 to DRB4*03:01N**, **DRB5*01:01:01 to DRB5*02:17**, recognized by the HLA Nomenclature Committee in October 2017^{1,2} 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.

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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*03:126, 14:179	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, 13:232	DRB1*13:44, 13:86, 13:206, 14:183
DRB1*08:31, 08:41, 08:75, 11:67, 11:193:01-11:193:02, 11:209	DRB1*13:235, 14:98

¹DRB alleles listed on the IMGT/HLA web page 2017-October-27, release 3.30.0, www.ebi.ac.uk/imgt/hla.

²Alleles 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	210 bp, 235 bp, 260 bp	515 bp	1	*01:01:01-01:02:13, 01:04-01:38, 01:40N-01:84
2	200 bp	430 bp	1/103	*01:03:01-01:03:02, 01:39N, 01:42, 01:61
3 ^{6,7,8}	200 bp, 230 bp	430 bp	2, 15	*15:01:01-01-15:147
4	210 bp	430 bp	11, 16	*11:30, 12:57, 16:01:01-16:05:02, 16:07-16:47
5 ^{5,11}	75 bp, 205 bp	515 bp	3, 11, 13, 17, 18	*03:01:01-01-03:125, 03:127-03:141, 11:07:01-11:07:02, 11:27:02-11:27:03, 11:84:02-11:84:03, 11:103:01-11:103:02, 11:105, 11:107, 11:125, 11:136, 11:173, 13:33:01, 13:61:02, 13:94:01, 13:96:01, 14:38:02, 14:104, 14:111, 14:171, 15:25, 15:37:01, 15:100, 15:104:01
6 ^{5,11}	75 bp, 205 bp	430 bp	3, 6, 11, 13, 14, 17	*03:01:01-01-03:01:23, 03:01:25-03:01:26, 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, 03:116-03:118, 03:121- 03:130, 03:132-03:141, 08:40, 11:02:01- 11:03:02, 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, 11:176, 11:182, 11:184, 11:213, 13:01:01-01-13:04, 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, 13:193-13:194, 13:196, 13:198,

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7⁵	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, 03:119, 03:126, 03:131, 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, 13:198, 13:206, 13:235, 14:02:01-14:03:02, 14:06:01-14:06:03, 14:09, 14:12:01-14:13, 14:17-14:21, 14:24, 14:27:01-14:27:02, 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, 14:159-14:160, 14:165, 14:170, 14:174, 14:176-14:177, 14:179, 14:181, 14:183
8⁵	100 bp, 175 bp	430 bp	3, 4	*04:01:01:01-04:05:11, 04:05:13-04:236
9⁷	190 bp, 210 bp, 235 bp	430 bp	7, 13, 14	*07:01:01:01-07:01:21, 07:03-07:81, 08:67, 10:11, 12:22, 13:17, 13:116, 13:175, 13:214, 14:50
10	170 bp, 215 bp, 250 bp	515 bp	7, 8, 11, 12, 14	*07:06, 08:01:01-08:01:02, 08:01:04-08:19, 08:21-08:56, 08:58-08:66, 08:68-08:79, 08:81-08:82, 11:67, 11:193:01-11:193:02, 11:209, 12:04, 12:16:01-12:16:03, 12:22, 12:39, 12:49, 13:171:01, 13:197, 14:11, 14:15, 14:68:01-14:68:02, 14:93, 14:148
11^{5,8,12}	85 bp, 100 bp, 135 bp, 160 bp, 190 bp	430 bp	3, 9, 11	*03:08, 03:65, 03:140, 09:01:02:01-09:32, 11:07:01-11:07:02, 11:103:01-11:103:02, 11:105, 11:107, 11:125, 11:173, DRB3*01:42
12	175 bp	430 bp	9, 10, 11, 13	*03:76, 03:125-03:126, 09:05, 10:01:01:01-10:22, 11:59, 11:80, 11:83, 11:87, 11:135, 11:142, 11:182, 13:27, 13:41, 13:71, 13:129, 13:176, 14:82, 14:95, 14:132, 14:179
13⁵	100 bp, 175 bp	430 bp	3, 8, 11, 13, 14	*03:08, 03:65, 03:140, 08:31, 08:41, 08:63, 08:75, 11:01:01:01-11:11:01, 11:11:03-11:70, 11:72-11:217N
14⁵	90 bp, 110 bp	430 bp	12, 14	*08:32, 08:53, 12:01:01:01-12:56, 12:58-12:68, 14:52
15¹²	220 bp	430 bp	6, 8, 11, 13, 14, 1403	*03:76, 03:125, 08:20-08:21, 11:01:01:01-11:01:27, 11:01:28 [?] , 11:01:29-11:04:15, 11:06:01-11:06:03, 11:08:01-11:11:01, 11:11:03-11:12:03, 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,

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16 ⁶	195 bp, 225 bp	430 bp	6, 8, 11, 12, 13, 14	*03:125, 08:01:01-08:01:02, 08:01:04-08:01:05, 08:02:01:01-08:02:04, 08:04:01-08:09, 08:11, 08:16-08:17, 08:20-08:22, 08:24:01-08:24:02, 08:26, 08:28, 08:31, 08:39, 08:41-08:44, 08:50, 08:52, 08:54-08:55, 08:57, 08:59, 08:64, 08:67, 08:70, 08:72-08:73, 08:75, 08:77-08:80, 08:82, 11:01:01:01-11:01:17, 11:01:20-11:06:03, 11:09-11:11:01, 11:11:03-11:12:03, 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, 11:65:01-11:70, 11:72, 11:74:01-11:78, 11:80-11:88, 11:90-11:97, 11:99-11:102:02, 11:106, 11:108-11:118, 11:120-11:124, 11:126-11:129, 11:133-11:135, 11:137-11:142, 11:144-11:152, 11:154-11:158, 11:160-11:172, 11:174-11:183, 11:185-11:190, 11:192-11:201, 11:203-11:217N, 12:02:01:01-12:02:07, 12:13, 12:15-12:16:03, 12:18-12:21, 12:23, 12:26-12:27, 12:31N-12:33, 12:37, 12:42-12:45, 12:49-12:52, 12:55-12:57, 12:60N-12:61, 12:64-12:65, 12:67, 13:01:01:01-13:02:01:03, 13:02:03-13:02:13, 13:04-13:05:03, 13:07:01-13:09, 13:11:01-13:11:02, 13:14:01-13:24, 13:26:01-13:29, 13:31-13:32, 13:34-13:36, 13:38-13:43, 13:45-13:55, 13:57, 13:59, 13:61:01-13:65, 13:67-13:76, 13:78-13:80, 13:83-13:84, 13:87, 13:91-13:93, 13:96:01-13:100, 13:102-13:109, 13:111-13:114, 13:116-13:117, 13:121, 13:123-13:132, 13:135-13:136, 13:138-13:150, 13:153, 13:155, 13:158-13:160, 13:162, 13:164-13:166, 13:168-13:169, 13:171:01-13:171:02, 13:173, 13:175, 13:177, 13:179, 13:182-13:187, 13:189-13:192, 13:195,

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17 ¹¹	175 bp, 240 bp	430 bp	3, 6, 8, 11, 13, 14, 1403, 1404, 17, 18	*03:01:01:01-03:01:05, 03:01:07-03:01:08, 03:01:10-03:07, 03:09, 03:11:01-03:41:02, 03:43-03:45, 03:47-03:63, 03:66-03:68N, 03:70-03:86, 03:88-03:91, 03:93-03:110, 03:112-03:129, 03:131-03:139, 03:141, 08:20, 11:13:01-11:13:02, 11:149, 11:202, 12:20, 13:01:01:01-13:16, 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, 13:171:02-13:174, 13:176-13:178, 13:180-13:190, 13:192-13:194, 13:196, 13:198-13:213, 13:215-13:216, 13:218-13:227, 13:229-13:240, 13:242-13:243, 14:01:01, 14:01:02 [?] -14:01:04 [?] , 14:02:01-14:04:01, 14:04:02 [?] -14:04:03 [?] , 14:05:01:01-14:07:01, 14:07:02 [?] , 14:08-14:14, 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 [?] , 14:27:01-14:27:02, 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 [?] , 14:54:01:01, 14:54:01:02 [?] , 14:54:02-14:54:06, 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 [?] -14:126:02 [?] , 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 [?] , 14:159-14:161, 14:162 [?] -14:164 [?] , 14:165, 14:166N [?] , 14:167, 14:168 [?] , 14:169-14:171, 14:172 [?] -14:173 [?] , 14:174, 14:175 [?] , 14:176-14:177, 14:178 [?] , 14:179, 14:180 [?] , 14:181, 14:182 [?] , 14:183, 14:184 [?] -14:186 [?] , 14:187-14:188N

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Lot-specific information

18^{5,10}	100 bp, 150 bp	430 bp	4, 6, 8,13, 14, 1404	*04:62, 04:69, 04:73:01-04:73:02, 04:105:01-04:105:02, 04:122, 04:146, 08:08, 11:69, 11:82, 13:45, 13:197, 14:01:01-14:01:02, 14:01:04, 14:04:01-14:04:03, 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, 14:53-14:54:01:02, 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, 14:163-14:164, 14:166N, 14:168 ^w , 14:171-14:173, 14:178, 14:180, 14:182, 14:184, 14:186, DRB4*01:03:01:02N
19^{5,6,9}	110 bp, 145 bp, 170 bp	430 bp	3, 4, 6, 9, 11, 13, 14, 1404	*03:10, 03:126, 04:211, 09:01:02:01-09:01:05, 09:01:07-09:02:02, 09:04-09:22, 09:24-09:28, 09:30-09:32, 11:13:01-11:13:02, 11:17, 11:52, 11:202, 12:66, 13:43, 13:159, 13:171:01, 13:179, 13:191, 13:193, 13:217, 14:01:01-14:02:03, 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, 14:54:01:01-14:54:03, 14:54:04 ^w , 14:54:05-14:57, 14:59-14:62, 14:64-14:65, 14:68:01-14:68:02, 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, 14:157-14:160, 14:162-14:173, 14:175-14:180, 14:182, 14:184-14:185, 14:187-14:188N, 15:27, 15:34, 15:54, 15:66:01-15:66:02, 15:122
20^{5,13}	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, 08:68 ^w , 08:70 ^w , 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, 11:202, 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, 13:197, 13:217, 13:232, 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, 14:160-14:164, 14:166N-14:169, 14:171-14:175, 14:178, 14:180-14:182, 14:184-14:188N, 15:21 ^w , 16:04:01 ^w -16:04:02 ^w , 16:18 ^w , DRB4*01:31

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

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Lot No.: **2G1**

Lot-specific information

21 ^{5,11,12}	80 bp, 160 bp, 240 bp	430 bp	52	*14:141, DRB3*01:01:02:01-01:45, DRB3*02:01-02:65, DRB3*03:01:01-03:13
22 ^{6,10,13}	130 bp, 215 bp	430 bp	53	*10:19, 15:141, DRB4*01:01:01:01-01:56N, DRB4*02:01N, DRB4*03:01N
23	175 bp	430 bp	51	DRB5*01:01:01-01:35, DRB5*02:02:01- 02:17
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.

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.

⁶Primer mixes 1, 3, 16, 19 and 22 have a tendency to giving rise to primer oligomer formation.

⁷Primer mixes 3 and 9 may have tendencies of unspecific amplification.

⁸Primer mixes 3 and 11 may give rise to a lower yield of HLA-specific PCR product than the other DR low primer mixes.

⁹Primer 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.

¹⁰The 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 only amplified by the primer pairs in well 22.

¹¹Due to sharing of sequence motifs in codon 38 and 47, DRB3*01:14 will also be amplified in primer mixes 5, 6, 17, and DRB3*01:23 and *02:32 will be amplified in primer mix 5, in addition to primer mix 21.

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

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Lot No.: 2G1

Lot-specific information

¹²Due to sharing of sequence motifs, DRB3*02:27 is amplified by the primer pairs in well 15, and DRB3*01:42 is amplified by the primer pairs in well 11, in addition to primer mix 21.

¹³Due to sharing of sequence motifs, DRB4*01:31 is amplified by the primer pairs in well 20 in addition to primer mix 22.

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.

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

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Lot No.: **2G1**

Lot-specific information
PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	210	200	200	210	75	75	85	100	170	170	85	175
PCR product	235		230		205	205	210	175	210	215	100	
	260								235	250	135	
											160	
											190	
Length of int. pos. control ¹	515	430	430	430	515	430	430	430	430	515	430	430
5'-primer(s) ²	13(124) 5'-A.T 3'	14(129) 5'-gAA 3'	12(121) 5'-CTg 3'	11(119) 5'-gCT 3'	13(125) 5'-gTC 3'	13(125) 5'-gTC 3'	13(125) 5'-gTC 3'	13(125) 5'-ACA 3'	11(118) 5'-AgA 3'	16(133) 5'-gTT 3'	26(165) 5'-TAT 3'	26(164) 5'-gTA 3'
	14(129) 5'-gAA 3'		13(126) 5'-AgA 3'	13(126) 5'-Agg 3'	47(227) 5'-gTT 3'	16(133) 5'-gTT 3'		13(125) 5'-ACC 3'	14(127) 5'-gTA 3'	16(133) 5'-gTT 3'	37(196) 5'-AgA 3'	31(178) 5'-gCg 3'
			13(126) 5'-AAg 3'	13(126) 5'-AAg 3'				13(125) 5'-ATA 3'	14(127) 5'-ATA 3'	16(133) 5'-gCT 3'	58(261) 5'-gAg 3'	
			13(126) 5'-Agg 3'					13(125) 5'-gTC 3'	14(127) 5'-ATA 3'	60(265) 5'-AgT 3'		
									16(133) 5'-gTT 3'			
									31(178) 5'-gCg 3'			
3'-primer(s) ³	67(286) 5'-gAg 3'	67(286) 5'-gAT 3'	67(286) 5'-gAT 3'	67(286) 5'-gAA 3'	73(305) 5'-ggC 3'	26(164) 5'-ggT 3'	28(171) 5'-CTC 3'	33(184) 5'-gTg 3'	71(298) 5'-CTC 3'	58(260) 5'-CCT 3'	57(257) 5'-CgA 3'	72(303) 5'-gCA 3'
	67(286) 5'-gAg 3'		67(286) 5'-gAT 3'	67(286) 5'-gAg 3'	73(305) 5'-ggC 3'	67(286) 5'-gAT 3'	70(295) 5'-CTg 3'	58(260) 5'-Cgg 3'	73(305) 5'-ggC 3'	74(307) 5'-CAg 3'	73(305) 5'-ggC 3'	74(307) 5'-CgC 3'
	67(286) 5'-gAT 3'		67(286) 5'-gAT 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'	78(319) 5'-CAC 3'	74(308) 5'-CCT 3'
	70(297) 5'-CTg 3'		70(295) 5'-Tg 3'	72(302) 5'-Cgg 3'	73(305) 5'-ggC 3'				78(319) 5'-CgC 3'			
	71(299) 5'-gCg 3'		70(295) 5'-CTg 3'		73(306) 5'-Tgg 3'				78(319) 5'-CAA 3'			
	77(317) 5'-AgT 3'		71(299) 5'-gCT 3'		74(308) 5'-CCC 3'				78(319) 5'-CAC 3'			
	86(344) 5'-CCA 3'		71(299) 5'-gCg 3'		75(310) 5'-CAA 3'							
			72(301) 5'-Cg 3'		77(317) 5'-AgT 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

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

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Lot No.: **2G1**

Lot-specific information

Well No.	13	14	15	16	17	18	19	20	21	22	23
Length of spec.	100	90	220	195	175	100	110	110	80	130	175
PCR product	175	110		225	240	150	145	150	160	215	
							170	180	240		
								220			
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'	13(124) 5'-Cgg 3'	10(116) 5'-gCT 3'	10(116) 5'-gCT 3'	13(125) 5'-gTC 3'	1 st 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'
	16(133) 5'-gTC 3'	16(133) 5'-gTT 3'	12(122) 5'-TAT 3'	12(122) 5'-TAT 3'	13(125) 5'-gTg 3'	37(197) 5'-gTT 3'	34(189) 5'-CAg 3'	34(189) 5'-CAg 3'	10(116) 5'-gCT 3'	28(170) 5'-gAg 3'	
	38(200) 5'-CgT 3'		13(125) 5'-gTC 3'	12(123) 5'-ACA 3'	114(429) 5'-CTg 3'	37(197) 5'-gTA 3'	34(189) 5'-CAg 3'	37(196) 5'-AgC 3'	38(199) 5'-TCC 3'	105(401) 5'-AAA 3'	
				13(125) 5'-gTC 3'							
				13(125) 5'-gTg 3'							
				16(133) 5'-gTC 3'							
				16(133) 5'-gTT 3'							
3'-primer(s) ³	58(260) 5'-CCT 3'	28(171) 5'-CTC 3'	70(295) 5'-gTC 3'	67(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'	87(346) 5'-CTC 3'	57(256) 5'-gTC 3'
	58(260) 5'-CCT 3'	30(175) 5'-gTg 3'	71(299) 5'-gCT 3'	71(298) 5'-CTC 3'	58(260) 5'-CAg 3'	57(257) 5'-CAg 3'	70(295) 5'-CTg 3'	60(265) 5'-gTg 3'	77(317) 5'-AAT 3'	87(346) 5'-CTT 3'	57(258) 5'-gCg 3'
	58(260) 5'-CCT 3'	38(199) 5'-CAg 3'	71(299) 5'-ACT 3'	71(298) 5'-CgC 3'	181(630) 5'-CTT 3'	71(298) 5'-CgC 3'	70(296) 5'-TCC 3'	70(296) 5'-TCC 3'		135(490) 5'-gCT 3'	58(260) 5'-CCT 3'
	58(261) 5'-TCT 3'		74(307) 5'-CgT 3'					74(307) 5'-CAg 3'			
			78(319) 5'-CAC 3'								
Well No.	13	14	15	16	17	18	19	20	21	22	23

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.: **2G1**

Lot-specific information

CELL LINE VALIDATION SHEET																					
DR low resolution primer set³																					
					Well²																
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
					Prod. No.:	201673401	201673402	201673403	201888904	201788005	201673406	201673407	201673408	201673409	201888910	201888911	201675112	201673413	201673414	201782715	201673416
IHWC cell line¹			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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“Instructions for Use” (IFU)

Lot No.: **2G1**

Lot-specific information

CELL LINE VALIDATION SHEET											
DR low resolution primer set ³											
				Well ²							
				17	18	19	20	21	22	23	
				Prod. No.:	201673417	201673418	201785419	201673420	201673429	201675130	201782731
	IHCW 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	PTOUT		*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-specific information

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.

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, 12, 15 and 18 were tested by separately adding one 3'-primer, respectively one or three 5'-primers.

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

Additional 3'-primers in primer solutions 1, 3, 5, 10 and 20 were tested by separately adding one or more 5'-primers.

One or more of the 5'-primers in primer solution 1, 3, 4, 8 to 10, 13 to 17, 20 and 22 were not possible to test. One or more of the 3'-primers in primer solution 1, 3 to 6, 9, 13, 15, 22 and 23 were not possible to test.

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