

101.125-24/06 – including *Taq* pol., IFU-01.
101.125-24u/06u – without *Taq* pol., IFU-02.

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“Instructions for Use” (IFU)

Lot No.: **2F2**

Lot-specific information
Olerup SSP[®] DRB1*15

Product number:	101.125-24/06 – including <i>Taq</i> pol. 101.125-24u/06u – without <i>Taq</i> pol.
Lot number:	2F2
Expiry date:	2019-12-01
Number of tests:	24 test – Product No. 101.125-24/24u 6 tests – Product No. 101.125-06/06u
Number of wells per test:	31+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. 2F2

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

**CHANGES COMPARED TO THE PREVIOUS OLERUP SSP[®]
DRB1*15 Lot (0D1)**

The DRB1*15 kit is updated for new alleles to enable separation of:

- Confirmed¹ alleles as listed in the IMGT/HLA database
- Polymorphisms in exons outside of the region encoding the peptide binding domain
- Null and Alternatively expressed alleles

The format of the Worksheet has been changed.

¹As described in section Uniquely Identified Alleles.

The DRB1*15 specificity and interpretation tables have been updated for the DRB1 alleles described since the previous *Olerup SSP[®]* DRB1*15 lot was made (**Lot No. 0D1**). The kit design is based on IMGT/HLA database 3.26.0.

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

Well	5'-primer	3'-primer	rationale
1	Modified	-	5'-primer modified for improved HLA-specific amplification.
2	Modified	-	5'-primer modified for improved HLA-specific amplification.
3	Added	-	5'-primer added for the DRB1*15:01:28 allele.
4	Modified	-	5'-primer modified for improved HLA-specific amplification.
5	Modified	-	5'-primer modified for improved HLA-specific amplification.
7	Modified	-	5'-primer modified for improved HLA-specific amplification.
9	Added	-	5'-primer added for the DRB1*15:138N allele.
12	-	Added	3'-primer added for the DRB1*15:123 allele.
15	-	Added	3'-primer added from well 31 for improved HLA-specific amplification.
16	-	Added	3'-primer added for the DRB1*15:129N allele.
17	-	Added	3'-primer added for the DRB1*15:123 allele.
22	Added	-	5'-primer added for the DRB1*15:138N allele.
26	Modified, added	-	5'-primer modified for improved HLA-specific amplification, 5'-primer added for the DRB1*15:134N allele.
29	-	Added	3'-primer added for the DRB1*15:137N allele.
30	-	Added	3'-primer added for the DRB1*15:129N allele.
31	Modified	Moved, modified	3'-primer moved to well 15, primer pair modified improved HLA-specific amplification.

Change in revision R01 compared to R00:

1. Primer mix 7 does not amplify the DRB1*15:56 allele. Thus, this lot of the DRB1*15 subtyping kit cannot distinguish the DRB1*15:56 and DRB1*15:01:01:01-15:01:31, 15:51-15:53, 15:62, 15:71-15:72, 15:76, 15:79, 15:81-15:83, 15:86-15:87, 15:90, 15:92, 15:95, 15:97-15:98, 15:106-15:109, 15:111, 15:114, 15:116, 15:121, 15:124, 15:127-15:128, 15:132-15:133, 15:135 and 15:139 alleles. This has been corrected in the Specificity and Interpretation Tables.

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Well **32** 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

DRB1*15 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*15:01 to DRB1*15:139 alleles.

PLATE LAYOUT

Each test consists of 32 PCR reactions in a 32 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
25	26	27	28	29	30	31	NC

The 32 well cut PCR plate is marked with 'DRB1*15' in silver/gray ink.

Well No. 1 is marked with the Lot No. '2F2'.

Wells 1 to 31 – DRB1*15 high resolution primers.

Well 32 – Negative Control (NC).

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 32 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

Due to the sharing of sequence motifs between DRB1 alleles, non-DRB1*15 alleles will be amplified by other primer mixes. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the DRB1*15 alleles, i.e. **DRB1*15:01 to DRB1*15:139**, recognized by the HLA Nomenclature Committee in October 2016^{1,2} will be amplified by the primers in the DRB1*15 subtyping kit³.

The DRB1*15 kit enables separation of the confirmed DRB1*15 alleles as listed in the IMGT/HLA database. An HLA allele is listed as confirmed by IMGT/HLA if it has been sequenced by more than a single laboratory or from multiple sources. Current allele confirmation status for DRB1*15 alleles is listed below.

The DRB1*15 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.

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The following DRB1*15 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix
DRB1*15:06:01-15:06:03, 15:110	9
DRB1*15:16, 15:35	28
DRB1*15:42, 15:64	27
DRB1*15:75, 15:134N	26

¹DRB1 alleles listed on the IMGT/HLA web page 2016-October-14, release 3.26.0, 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>.

³The DRB1*15:70, 15:89 and 15:91 and the DRB1*16:33 and 16:36 alleles give rise to identical amplification patterns with the DRB1*15 high resolution kit. These alleles can be distinguished by the DR low resolution and/or DRB1*16 kits.

⁴This lot of the DRB1*15 subtyping kit cannot distinguish the DRB1*15:56 and DRB1*15:01:01:01-15:01:31, 15:51-15:53, 15:62, 15:71-15:72, 15:76, 15:79, 15:81-15:83, 15:86-15:87, 15:90, 15:92, 15:95, 15:97-15:98, 15:106-15:109, 15:111, 15:114, 15:116, 15:121, 15:124, 15:127-15:128, 15:132-15:133, 15:135 and 15:139 alleles.

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ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
DRB1*15:01:01:01	Confirmed	DRB1*15:02:16	Unconfirmed	DRB1*15:42	Unconfirmed	DRB1*15:91	Unconfirmed
DRB1*15:01:01:02	Unconfirmed	DRB1*15:02:17	Unconfirmed	DRB1*15:43	Unconfirmed	DRB1*15:92	Unconfirmed
DRB1*15:01:01:03	Unconfirmed	DRB1*15:02:18	Unconfirmed	DRB1*15:44	Unconfirmed	DRB1*15:93	Confirmed
DRB1*15:01:01:04	Unconfirmed	DRB1*15:03:01:01	Confirmed	DRB1*15:45	Unconfirmed	DRB1*15:94	Confirmed
DRB1*15:01:02	Confirmed	DRB1*15:03:01:02	Confirmed	DRB1*15:46	Unconfirmed	DRB1*15:95	Unconfirmed
DRB1*15:01:03	Confirmed	DRB1*15:03:02	Confirmed	DRB1*15:47	Unconfirmed	DRB1*15:96	Unconfirmed
DRB1*15:01:04	Confirmed	DRB1*15:04	Confirmed	DRB1*15:48	Unconfirmed	DRB1*15:97	Unconfirmed
DRB1*15:01:05	Unconfirmed	DRB1*15:05	Confirmed	DRB1*15:49	Unconfirmed	DRB1*15:98	Unconfirmed
DRB1*15:01:06	Confirmed	DRB1*15:06:01	Confirmed	DRB1*15:50N	Unconfirmed	DRB1*15:99	Unconfirmed
DRB1*15:01:07	Confirmed	DRB1*15:06:02	Unconfirmed	DRB1*15:51	Unconfirmed	DRB1*15:100	Unconfirmed
DRB1*15:01:08	Confirmed	DRB1*15:06:03	Unconfirmed	DRB1*15:52	Unconfirmed	DRB1*15:101	Unconfirmed
DRB1*15:01:09	Unconfirmed	DRB1*15:07:01	Confirmed	DRB1*15:53	Unconfirmed	DRB1*15:102	Confirmed
DRB1*15:01:10	Unconfirmed	DRB1*15:07:02	Unconfirmed	DRB1*15:54	Unconfirmed	DRB1*15:103	Unconfirmed
DRB1*15:01:11	Confirmed	DRB1*15:07:03	Unconfirmed	DRB1*15:55	Confirmed	DRB1*15:104	Unconfirmed
DRB1*15:01:12	Confirmed	DRB1*15:08	Unconfirmed	DRB1*15:56	Confirmed	DRB1*15:105	Unconfirmed
DRB1*15:01:13	Confirmed	DRB1*15:09	Confirmed	DRB1*15:57	Confirmed	DRB1*15:106	Unconfirmed
DRB1*15:01:14	Unconfirmed	DRB1*15:10	Confirmed	DRB1*15:58	Unconfirmed	DRB1*15:107	Unconfirmed
DRB1*15:01:15	Unconfirmed	DRB1*15:11	Confirmed	DRB1*15:59	Unconfirmed	DRB1*15:108	Unconfirmed
DRB1*15:01:16	Unconfirmed	DRB1*15:12	Unconfirmed	DRB1*15:60	Unconfirmed	DRB1*15:109	Unconfirmed
DRB1*15:01:17	Unconfirmed	DRB1*15:13	Unconfirmed	DRB1*15:61	Confirmed	DRB1*15:110	Unconfirmed
DRB1*15:01:18	Unconfirmed	DRB1*15:14	Confirmed	DRB1*15:62	Unconfirmed	DRB1*15:111	Unconfirmed
DRB1*15:01:19	Unconfirmed	DRB1*15:15:01	Confirmed	DRB1*15:63	Unconfirmed	DRB1*15:112	Confirmed
DRB1*15:01:20	Unconfirmed	DRB1*15:15:02	Unconfirmed	DRB1*15:64	Confirmed	DRB1*15:113N	Confirmed
DRB1*15:01:21	Unconfirmed	DRB1*15:16	Unconfirmed	DRB1*15:65	Unconfirmed	DRB1*15:114	Unconfirmed
DRB1*15:01:22	Unconfirmed	DRB1*15:17N	Unconfirmed	DRB1*15:66:01	Unconfirmed	DRB1*15:115N	Unconfirmed
DRB1*15:01:23	Unconfirmed	DRB1*15:18	Confirmed	DRB1*15:66:02	Unconfirmed	DRB1*15:116	Unconfirmed
DRB1*15:01:24	Unconfirmed	DRB1*15:19	Unconfirmed	DRB1*15:67	Confirmed	DRB1*15:117	Unconfirmed
DRB1*15:01:25	Confirmed	DRB1*15:20	Confirmed	DRB1*15:68	Confirmed	DRB1*15:118	Confirmed
DRB1*15:01:26	Unconfirmed	DRB1*15:21	Unconfirmed	DRB1*15:69	Unconfirmed	DRB1*15:119	Unconfirmed
DRB1*15:01:27	Unconfirmed	DRB1*15:22	Confirmed	DRB1*15:70	Unconfirmed	DRB1*15:120	Unconfirmed
DRB1*15:01:28	Unconfirmed	DRB1*15:23	Confirmed	DRB1*15:71	Unconfirmed	DRB1*15:121	Unconfirmed
DRB1*15:01:29	Unconfirmed	DRB1*15:24	Confirmed	DRB1*15:72	Unconfirmed	DRB1*15:122	Unconfirmed
DRB1*15:01:30	Unconfirmed	DRB1*15:25	Unconfirmed	DRB1*15:73	Unconfirmed	DRB1*15:123	Confirmed
DRB1*15:01:31	Unconfirmed	DRB1*15:26	Unconfirmed	DRB1*15:74	Confirmed	DRB1*15:124	Unconfirmed
DRB1*15:02:01:01	Confirmed	DRB1*15:27	Confirmed	DRB1*15:75	Confirmed	DRB1*15:125	Unconfirmed
DRB1*15:02:01:02	Unconfirmed	DRB1*15:28	Unconfirmed	DRB1*15:76	Unconfirmed	DRB1*15:126	Unconfirmed
DRB1*15:02:02	Confirmed	DRB1*15:29	Unconfirmed	DRB1*15:77	Unconfirmed	DRB1*15:127	Unconfirmed
DRB1*15:02:03	Unconfirmed	DRB1*15:30	Unconfirmed	DRB1*15:78	Confirmed	DRB1*15:128	Unconfirmed
DRB1*15:02:04	Confirmed	DRB1*15:31	Unconfirmed	DRB1*15:79	Unconfirmed	DRB1*15:129N	Unconfirmed
DRB1*15:02:05	Unconfirmed	DRB1*15:32	Unconfirmed	DRB1*15:80N	Unconfirmed	DRB1*15:130	Unconfirmed
DRB1*15:02:06	Unconfirmed	DRB1*15:33	Confirmed	DRB1*15:81	Unconfirmed	DRB1*15:131	Unconfirmed
DRB1*15:02:07	Unconfirmed	DRB1*15:34	Unconfirmed	DRB1*15:82	Unconfirmed	DRB1*15:132	Unconfirmed
DRB1*15:02:08	Unconfirmed	DRB1*15:35	Confirmed	DRB1*15:83	Unconfirmed	DRB1*15:133	Unconfirmed
DRB1*15:02:09	Unconfirmed	DRB1*15:36	Confirmed	DRB1*15:84	Unconfirmed	DRB1*15:134N	Unconfirmed
DRB1*15:02:10	Unconfirmed	DRB1*15:37:01	Confirmed	DRB1*15:85	Unconfirmed	DRB1*15:135	Unconfirmed
DRB1*15:02:11	Unconfirmed	DRB1*15:37:02	Confirmed	DRB1*15:86	Unconfirmed	DRB1*15:136	Confirmed
DRB1*15:02:12	Unconfirmed	DRB1*15:38	Confirmed	DRB1*15:87	Unconfirmed	DRB1*15:137N	Unconfirmed
DRB1*15:02:13	Unconfirmed	DRB1*15:39	Unconfirmed	DRB1*15:88	Unconfirmed	DRB1*15:138N	Unconfirmed
DRB1*15:02:14	Unconfirmed	DRB1*15:40	Confirmed	DRB1*15:89	Unconfirmed	DRB1*15:139	Unconfirmed
DRB1*15:02:15	Confirmed	DRB1*15:41	Unconfirmed	DRB1*15:90	Unconfirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2016-October-14, release 3.26.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DRB1*15 homo- and heterozygotes is available upon request.

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Lot-specific information
SPECIFICITY TABLE

DRB1*15 SSP subtyping

Specificities and sizes of the PCR products of the 31+1 primer mixes used for DRB1*15 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB1*15 alleles ³	Other amplified DRB1 alleles
1	140 bp	515 bp	*15:01:01:01-15:02:09, 15:02:11-15:06:03, 15:08-15:10, 15:12-15:27, 15:29-15:33, 15:35-15:47, 15:49-15:58, 15:60-15:68, 15:70-15:87, 15:89-15:95, 15:97-15:114, 15:116-15:129N, 15:131-15:139	*16:09:01-16:10, 16:33, 16:36-16:37
2	200 bp	515 bp	*15:01:01:01-15:03:02, 15:06:01-15:09, 15:11-15:14, 15:16, 15:18 ^w , 15:19-15:20, 15:22, 15:24, 15:26, 15:29-15:30, 15:32-15:33, 15:35-15:48, 15:51-15:53, 15:55-15:62, 15:64-15:65, 15:67-15:68, 15:71-15:72, 15:74-15:79, 15:81-15:83, 15:85-15:87, 15:90, 15:92-15:99, 15:101-15:102, 15:104-15:114, 15:116, 15:118-15:119, 15:121, 15:123-15:128, 15:130-15:139	
3	150 bp	430 bp	*15:01:01:01-15:02:18, 15:06:01-15:09, 15:11-15:14, 15:16, 15:18 ^w , 15:19-15:20, 15:22, 15:24, 15:26, 15:28-15:30, 15:32-15:33, 15:35-15:48, 15:51-15:53, 15:55-15:56, 15:59-15:62, 15:64-15:65, 15:67-15:69, 15:71-15:72, 15:74-15:77, 15:79, 15:81-15:83, 15:85-15:87, 15:90, 15:92-15:93, 15:95-15:99, 15:101-15:102, 15:104-15:114, 15:116, 15:118-15:119, 15:121, 15:123-15:128, 15:131-15:136, 15:138N-15:139	*04:73, 04:105:01-04:105:02, 11:82, 13:09, 13:129, 14:37
4	260 bp	430 bp	*15:01:01:01-15:01:31, 15:03:01:01-15:07:03, 15:09-15:10, 15:12-15:13, 15:16-15:18, 15:20-15:25, 15:32-15:33, 15:35-15:37:02, 15:40-15:43, 15:45-15:46, 15:48-15:57, 15:59, 15:61-15:62, 15:64-15:67, 15:70-15:73, 15:75-15:77, 15:79, 15:81-15:84, 15:86-15:98, 15:100, 15:102, 15:106-15:114, 15:116-15:117, 15:120-15:121, 15:123-15:125, 15:127-15:130, 15:132-15:139	*16:15, 16:23, 16:33-16:34, 16:36, 16:38
5	260 bp	430 bp	*15:02:01:01-15:02:18, 15:08, 15:11, 15:14-15:15:02, 15:19, 15:26-15:27, 15:29-15:31, 15:34, 15:38-15:39, 15:44, 15:47, 15:58, 15:60, 15:63, 15:68, 15:78, 15:80N, 15:99, 15:101, 15:103-15:105, 15:115N, 15:118-15:119, 15:122, 15:126, 15:131	*16:01:01-16:01:06, 16:01:08-16:05:02, 16:07-16:11, 16:13N-16:14, 16:16-16:22, 16:24-16:32, 16:35, 16:37, 16:39-16:44
6	150 bp 180 bp	430 bp	*15:03:01:01-15:03:02, 15:57, 15:78, 15:94, 15:130, 15:137N *15:14	

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7	135 bp	430 bp	*15:07:01-15:07:03, 15:11, 15:34, 15:88, 15:96, 15:115N	*16:01:01-16:05:02, 16:07-16:08, 16:11, 16:13N-16:32, 16:34-16:35, 16:38- 16:43
8	200 bp 245 bp	430 bp	*15:05, 15:23, 15:25, 15:27, 15:31, 15:34, 15:54, 15:63, 15:66:01-15:66:02, 15:73, 15:100, 15:120 *15:36	
9⁴	90 bp 165 bp 215 bp	430 bp	*15:06:01-15:06:03, 15:77, 15:94, 15:125 *15:138N *15:19, 15:69, 15:110, 15:113N	
10	170 bp 200 bp	430 bp	*15:12 *15:04, 15:15:01-15:15:02, 15:88	
11	155 bp 200 bp	430 bp	*15:47, 15:65 *15:49	
12⁴	95 bp 220 bp	430 bp	*15:09, 15:48 *15:123	*04:162, 11:88, 13:177, 16:44
13⁴	110 bp 215 bp	430 bp	*15:32 *15:10, 15:84, 15:103, 15:117, 15:120	
14	200 bp	430 bp	*15:13, 15:17N	
15	150 bp 205 bp	515 bp	*15:68, 15:136 *15:10, 15:21, 15:27, 15:34, 15:54, 15:66:01-15:66:02, 15:122	*16:05:01-16:05:02, 16:07
16⁴	110 bp 210 bp 260 bp	430 bp	*15:102 *15:08, 15:129N *15:74	
17	160 bp 220 bp	430 bp	*15:18 *15:123	
18	160 bp 205 bp	515 bp	*15:115N *15:20	
19⁶	175 bp 225 bp	430 bp	*15:30 *15:21, 15:25, 15:37:01-15:37:02, 15:57, 15:100, 15:104	*16:04, 16:18
20	165 bp 200 bp	430 bp	*15:22 *15:25, 15:27, 15:34, 15:54, 15:66:01- 15:66:02, 15:100, 15:112, 15:120	
21	165 bp	430 bp	*15:03:01:01-15:03:02, 15:23, 15:78, 15:94, 15:130, 15:137N	
22⁵	170 bp 200 bp	430 bp	*15:24, 15:93, 15:138N *15:28, 15:40	
23⁴	125 bp 170 bp 220 bp	430 bp	*15:55 *15:29 *15:25	
24	140 bp 200 bp	430 bp	*15:61 *15:26, 15:40, 15:43	
25^{4,6}	70 bp 135 bp 195 bp	430 bp	*15:39 *15:61, 15:67 *15:33, 15:113N	
26^{4,6}	75 bp 125 bp 195 bp	430 bp	*15:38, 15:63 *15:75 *15:134N	

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27^{4,6}	110 bp 135 bp 175 bp	430 bp	*15:64 *15:67 *15:14, 15:42, 15:93
28⁶	135 bp 255 bp	430 bp	*15:16, 15:118 *15:35, 15:96
29⁴	100 bp 200 bp	430 bp	*15:137N *15:41, 15:50N, 15:80N, 15:112
30	165 bp 215 bp	430 bp	*15:45 *15:44, 15:129N
31⁴	85 bp	515bp	*15:46
32⁷	-	-	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 DRB1*15 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.

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.

³For several DRB1 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.

⁴HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁵Primer mix 22 may have a tendency of unspecific amplification.

⁶Primer mixes 19, 25, 26, 27 and 28 have a tendency to giving rise to primer oligomer formation.

⁷Primer mix 32 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’, may be weakly amplified.

101.125-24/06 – including *Taq* pol., IFU-01.
101.125-24u/06u – without *Taq* pol., IFU-02.

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Lot No.: **2F2**

Lot-specific information

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	140	200	150	260	260	150	135	200	90	170	155	95
						180		245	165	200	200	220
									215			
Length of int. pos. control ¹	515	515	430	430	430	430	430	430	430	430	430	430
5'-primer(s) ²	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	29(174) 5'-Agg 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	20(146) 5'-TgC 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	5(103) 5'-CAg 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	29(175) 5'-gAT 3'
	13(126) 5'-AgA 3'	13(126) 5'-AgA 3'	29(175) 5'-gAT 3'	13(126) 5'-AgA 3'		29(175) 5'-gAC 3'			9(115) 5'-ggT 3'			
									11(121) 5'-CTg 3'			
									24(160) 5'-TgC 3'			
									50(236) 5'-ggg 3'			
									50(236) 5'-ggC 3'			
3'-primer(s) ³	47(227) 5'-ggA 3'	66(286) 5'-gAT 3'	66(286) 5'-gAT 3'	86(344) 5'-CCA 3'	86(344) 5'-CAC 3'	66(286) 5'-gAT 3'	44(218) 5'-CCC 3'	66(286) 5'-gAg 3'	66(286) 5'-gAT 3'	56(256) 5'-gCT 3'	51(239) 5'-CCC 3'	48(230) 5'-CCT 3'
							47(227) 5'-ggT 3'	66(286) 5'-gAg 3'		66(286) 5'-gAA 3'	67(287) 5'-ggg 3'	90(356) 5'-CTA 3'
								81(329) 5'-TgC 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	24
Length of spec. PCR product	110	200	150	110	160	160	175	165	165	170	125	140
	215		205	210	220	205	225	200		200	170	200
				260							220	
Length of int. pos. control ¹	430	430	515	430	430	515	430	430	430	430	430	430
5'-primer(s) ²	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	29(175) 5'-gAT 3'	29(175) 5'-gAT 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	29(175) 5'-gAC 3'	13(126) 5'-AAg 3'	13(126) 5'-Agg 3'	15(131) 5'-gTA 3'
										15(131) 5'-gTA 3'		19(143) 5'-CAg 3'
										22(154) 5'-AgT 3'		20(148) 5'-ggT 3'
										24(160) 5'-TgC 3'		37(199) 5'-CCA 3'
										25(161) 5'-gCA 3'		
3'-primer(s) ³	36(196) 5'-ggT 3'	63(277) 5'-Tg 3'	49(233) 5'-CCT 3'	36(196) 5'-gAg 3'	69(295) 5'-CTC 3'	69(293) 5'-CT 3'	57(258) 5'-gCC 3'	55(251) 5'-gCA 3'	70(298) 5'-CgC 3'	66(286) 5'-gAT 3'	40(208) 5'-gTg 3'	70(298) 5'-CgC 3'
	71(299) 5'-gCT 3'	69(294) 5'-TgT 3'	66(286) 5'-gAT 3'	65(283) 5'-gTT 3'	90(356) 5'-CTA 3'	83(337) 5'-CCT 3'	73(307) 5'-CAg 3'	65(282) 5'-TCA 3'			57(257) 5'-CgA 3'	
			66(286) 5'-gAT 3'	71(301) 5'-Cg 3'			77(317) 5'-AgT 3'	66(286) 5'-gAg 3'			73(305) 5'-ggC 3'	
			70(296) 5'-TCT 3'	87(347) 5'-TCC 3'			77(317) 5'-AAT 3'					
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

101.125-24/06 – including *Taq* pol., IFU-01.
101.125-24u/06u – without *Taq* pol., IFU-02.

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Lot No.: **2F2**

Lot-specific information

Well No.	25	26	27	28	29	30	31
Length of spec.	70	75	110	135	100	165	85
PCR product	135	125	135	255	200	215	
	195	195	175				
Length of int.	430	430	430	430	430	430	515
pos. control ¹							
5'-primer(s) ²	9(115) 5'-ggT 3'	19(144) 5'-.T 3'	20(146) 5'-TgC 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	13(126) 5'-Agg 3'	71(301) 5'-Cgg 3'
	14(130) 5'-AgA 3'	43(216) 5'-gAg 3'	22(154) 5'-AgT 3'				
	36(194) 5'-ggg 3'	60(266) 5'-gTC 3'	23(155) 5'-gCA 3'				
	37(199) 5'-CCA 3'		36(194) 5'-ggg 3'				
	57(259) 5'-ACA 3'		43(217) 5'-ACA 3'				
3'-primer(s) ³	66(286) 5'-gAT 3'	70(298) 5'-CgC 3'	66(286) 5'-gAT 3'	44(220) 5'-CCT 3'	33(187) 5'-CTA 3'	54(250) 5'-CCA 3'	86(344) 5'-CCA 3'
				46(226) 5'-gAg 3'	62(273) 5'-CTC 3'	71(301) 5'-.Cg 3'	
				85(341) 5'-CAg 3'	65(282) 5'-TCA 3'	73(305) 5'-CgA 3'	
					69(295) 5'-.Tg 3'		
Well No.	25	26	27	28	29	30	31

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.

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101.125-24u/06u – without *Taq* pol., IFU-02.

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Lot No.: **2F2**

Lot-specific information

CELL LINE VALIDATION SHEET																				
DRB1*15 SSP subtyping kit ²																				
				Well																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Prod. No.:	201780101	201780102	201780103	201780104	201780105	201436306	201780107	201436308	201780109	201436310	201436332	201780112	201780113	201780114	201780115	201780116
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 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	*14:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

101.125-24/06 – including *Taq* pol., IFU-01.
 101.125-24u/06u – without *Taq* pol., IFU-02.

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Lot No.: **2F2**

Lot-specific information

CELL LINE VALIDATION SHEET																							
DRB1*15 SSP subtyping kit ²																							
				Well																			
				17	18	19	20	21	22	23	24	25	26	27	28	29	30	31					
				Prod. No.:	201780117	201780118	201436319	201558220	201436321	201780122	201436323	201436324	201558225	201780126	201558227	201558228	201780129	201780130	201780131				
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	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	*14:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
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		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				

101.125-24/06 – including *Taq* pol., IFU-01.
 101.125-24u/06u – without *Taq* pol., IFU-02.

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Lot No.: 2F2

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 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.

No DNAs carrying the alleles to be amplified by primer solutions 8, 9, 11 to 20 and 22 to 31 were available. The specificities of the primers in primer solutions 8, 11 to 13, 15, 16, 19, 20, 22, 23 and 28 were tested by separately adding one or three 5'-primers, respectively one 3'-primer. In primer solutions 14, 17, 18, 29 and 30 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 9, 24 to 27 and 31 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 3, 6 and 22 one or more of the 5'-primers were not possible to test, and in primer solutions 2, 3, 7, 8, 11 to 13, 15, 16, 19, 20, 23 and 28 one, two or three of the 3'-primers were not possible to test.

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Lot No.: **2F2**

Lot-specific information

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