

101.113-24/04 – including *Taq* polymerase, IFU-01
101.113-24u/04u – without *Taq* polymerase, IFU-02

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

Lot No.: **40Y**

Lot-specific information
Olerup SSP® DRB1*03

Product number:	101.113-24/04 – including <i>Taq</i> pol. 101.113-24u/04u – without <i>Taq</i> pol.
Lot number:	40Y
Expiry date:	2017-November-01
Number of tests:	24 tests – Product No. 101.113-24/24u 4 tests – Product No. 101.113-04/04u
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. 40Y.

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*03 Lot (52V)**

The DRB1*03 kit is updated to enable separation of:

- Confirmed DRB1*03 alleles as listed in the IMGT/HLA database¹
- Polymorphisms in exons outside of the region encoding the peptide binding domain
- Null and Alternatively expressed alleles

A well containing Negative Control primer pairs has been added.

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

¹As described in section Uniquely Identified Alleles.

The DRB1*03 primer set, specificity and interpretation tables have been updated for the DRB alleles described since the previous *Olerup SSP®* DRB1*03 lot was made (**Lot No. 52V**). The kit design is based on IMGT/HLA database 3.19.0.

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, added or modified compared to the previous lot.

Well	5'-primer	3'-primer	rationale
3	-	Added	3'-primers added for the DRB1*03:97 allele.
6	-	Added	3'-primers added for the DRB1*03:01:23 allele.
12	-	Added	3'-primers added for the DRB1*03:110 allele.
14	-	Added	3'-primers added for the DRB1*03:110 allele.
32	-	-	Updated negative control.

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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*03 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*03:01 to DRB1*03:118 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*03' in silver/gray ink.

Well No. 1 is marked with the Lot No. '40Y'.

Wells 1 to 31 – DRB1*03 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 heat-sealed with a PCR-compatible foil.

Please note: When removing each 32 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Due to the sharing of sequence motifs within the DR52 group of DRB1 alleles, non-DRB1*03 alleles are amplified by primer mixes 1 to 4, 6 to 18, 20, 21, 24, 26, 27, 29 to 31. In addition, a few DRB3 alleles will be amplified by primer mixes 3, 8 to 11 and 24 to 27.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the phenotypically different DRB1*03 alleles, i.e. **DRB1*03:01 to DRB1*03:118**, recognized by the HLA Nomenclature Committee in January 2015^{1,2} will be amplified by the primers in the DRB1*03 subtyping kit³.

The DRB1*03 kit enables separation of the confirmed DRB1*03 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*03 alleles is listed below.

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The DRB1*03 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.

The following DRB1*03 alleles can be distinguished by the different sizes of the specific PCR product:

Alleles	Primer mix
DRB1*03:18, 03:48	22
DRB1*03:20, 03:93	17

The DRB1*03 subtyping kit cannot distinguish the following silent mutations: the DRB1*03:01:01:01-03:01:24, the DRB1*03:02:01-03:02:02, the DRB1*03:04:01-03:04:02, the DRB1*03:05:01-03:05:03 or the DRB1*03:13:01-03:13:02 alleles.

¹DRB1 alleles listed on the IMGT/HLA web page 2014-January-17, release 3.15.0, 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*03 primer set cannot separate the DRB1*03:77, 03:84, 03:89, 03:95 and the DRB1*13:144 alleles. These alleles can be distinguished by the DR low resolution kit and/or the DRB1*13 high resolution kit.

The DRB1*03 primer set cannot separate the DRB1*03:97 and the 14:33 alleles. These alleles can be distinguished by the DR low resolution kit and/or the DRB1*14high resolution kit.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DRB1*03 homo- and heterozygotes is available upon request.

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ALLELE CONFIRMATION STATUS

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

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2015-January-19, release 3.19.0, www.ebi.ac.uk/imgt/hla.

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SPECIFICITY TABLE

DRB1*03 SSP subtyping

Specificities and sizes of the PCR products of the 31+1 primer mixes used for DRB1*03 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB1*03 alleles ³	Other amplified DRB alleles ⁴
1	220 bp	515 bp	*03:01:01:01-03:01:24, 03:04:01-03:04:02, 03:06, 03:08, 03:10-03:13:02, 03:15, 03:16 [?] , 03:18- 03:20, 03:22-03:23, 03:25-03:26, 03:28, 03:30-03:34, 03:36-03:37, 03:39, 03:42-03:48, 03:50-03:52, 03:55-03:56, 03:58-03:62, 03:64-03:73, 03:75-03:87, 03:89, 03:91-03:96, 03:98- 03:100:02, 03:104, 03:106-03:112, 03:114, 03:116-03:118	*09:06, 11:59, 11:83, 11:135, 13:27, 13:71, 13:129, 13:144, 13:176, 14:82, 14:95, 14:132
2^{5,8}	85 bp	430 bp	*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	*13:15, 13:19, 13:26:01-13:26:02, 13:53, 13:57, 13:85-13:86, 13:104, 14:02:01- 14:03:02, 14:06:01-14:06:03, 14:12:01- 14:13, 14:18-14:20, 14:24, 14:27, 14:29, 14:40-14:41, 14:47-14:49, 14:51, 14:63, 14:67, 14:77-14:78, 14:81, 14:83, 14:85, 14:89, 14:94, 14:98, 14:102, 14:106, 14:108-14:109, 14:115, 14:135, 14:154, 14:156, 14:159
3	155 bp 190 bp	515 bp	*03:97 *03:02:01-03:02:02, 03:05:01-03:05:03, 03:09, 03:14, 03:27, 03:29, 03:38, 03:53, 03:74, 03:81, 03:88, 03:90, 03:102-03:103, 03:115	*14:18, 14:33, 14:51, 14:59, 14:64, 14:95, 14:106, 14:132-14:134, 14:154 *11:09, 11:20, 11:28:02, 11:87, 11:122, 13:02:01-13:02:03, 13:02:05-13:02:11, 13:05:01-13:05:02, 13:26:01-13:26:02, 13:29, 13:31, 13:34, 13:36, 13:39, 13:41, 13:50:02-13:50:03, 13:56, 13:63, 13:65, 13:67, 13:73-13:74, 13:85, 13:96:01- 13:96:02, 13:99, 13:103-13:104, 13:119- 13:120, 13:123-13:124, 13:126, 13:128, 13:136, 13:139-13:140, 13:142N-13:143, 13:145, 13:147, 13:155, 13:157-13:159, 13:165, 13:168, 13:170-13:171:02, 13:179, 13:189, 13:196, 14:02:01-14:03:02, 14:09, 14:13, 14:19, 14:24, 14:27, 14:30, 14:46- 14:48, 14:51, 14:63, 14:67, 14:85, 14:89, 14:98, 14:109, 14:115, 14:134-14:135, 14:144, 16:08, DRB3*01:08, DRB3*02:06, DRB3*02:20

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4	190 bp	430 bp	*03:01:01:01-03:01:24, 03:03, 03:06-03:08, 03:10-03:13:02, 03:15, 03:16 [?] , 03:18-03:20, 03:22-03:24, 03:26, 03:28, 03:32-03:34, 03:36-03:37, 03:39, 03:42, 03:45-03:48, 03:50-03:51, 03:54-03:56, 03:58-03:62, 03:64-03:73, 03:76-03:87, 03:89, 03:91-03:101, 03:104, 03:106-03:114, 03:116- 03:117	*11:16, 11:40, 11:58:01, 11:59, 11:83, 11:113, 11:115, 13:01:01-13:01:16, 13:06, 13:09-13:10, 13:15, 13:18, 13:20, 13:27- 13:28, 13:32, 13:35, 13:40, 13:42-13:43, 13:51, 13:53, 13:59, 13:61:01-13:61:02, 13:69, 13:71, 13:77-13:80, 13:87, 13:91- 13:93, 13:98, 13:102, 13:105, 13:110- 13:114, 13:117, 13:121, 13:125, 13:127, 13:129-13:131, 13:137N-13:138, 13:141, 13:144, 13:146, 13:148, 13:153, 13:156, 13:160, 13:163, 13:166, 13:173, 13:176- 13:178, 13:181, 13:183-13:187, 13:190- 13:193, 14:06:01-14:06:03, 14:12:01- 14:12:02, 14:17-14:18, 14:21, 14:29, 14:33, 14:52, 14:59, 14:64, 14:78, 14:80-14:81, 14:83, 14:95, 14:106, 14:108, 14:121, 14:132-14:133, 14:154, 15:32
5⁵	75 bp 165 bp	430 bp	*03:04:01-03:04:02 *03:58, 03:65	
6⁵	105 bp	430 bp	*03:01:01:01-03:01:24, 03:04:01-03:05:03, 03:08- 03:15, 03:16 ^w , 03:18- 03:20, 03:22-03:23, 03:25, 03:28, 03:30, 03:32-03:34, 03:36-03:37, 03:39, 03:42-03:48, 03:50-03:52, 03:55-03:59, 03:61-03:73, 03:76-03:79, 03:81, 03:83-03:84, 03:86-03:87, 03:89, 03:91-03:96, 03:98- 03:100:02, 03:104, 03:106-03:112, 03:114, 03:116-03:118	*11:59, 11:80, 11:87, 11:135, 11:142, 13:27, 13:41, 13:71, 13:129, 13:144, 13:176, 14:95, 14:132
7	130 bp	430 bp	*03:08, 03:65, 03:72	*11:59, 11:80, 11:83, 11:87, 11:135, 11:142
8	185 bp	430 bp	*03:02:01-03:03, 03:07, 03:21, 03:29, 03:38, 03:40-03:41, 03:49, 03:53, 03:74, 03:88, 03:101-03:103, 03:105, 03:115	*01:16, 01:55, 04:22, 11:07, 11:103, 11:107, 11:125, 11:173, 14:76, 14:79, 15:25, DRB3*01:09, DRB3*02:04, DRB3*02:19, DRB3*02:22, DRB3*03:03
9	135 bp	430 bp	*03:01:01:01-03:01:24, 03:04:01-03:06, 03:09, 03:11:01, 03:13:01 ^w - 03:13:02 ^w , 03:14-03:16, 03:18-03:20, 03:22-03:23, 03:25-03:26, 03:28, 03:30-03:34, 03:36-03:37, 03:39, 03:42-03:45, 03:47-03:48, 03:50-03:52, 03:55-03:63, 03:66, 03:67N ^w , 03:68N-03:73, 03:75-03:78, 03:80-03:84, 03:86-03:87, 03:89,	*09:01:08, 09:02:01-09:02:02, 13:27, 13:41, 13:71, 13:129, 13:144, 13:176, 14:95, DRB3*01:07, DRB3*01:15

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			03:91-03:95, 03:98-03:100:02, 03:104, 03:106-03:109, 03:111-03:112, 03:114, 03:116, 03:117 ^w , 03:118	
10	145 bp	430 bp	*03:02:01-03:03, 03:06, 03:17, 03:26-03:27, 03:31, 03:35, 03:38, 03:41, 03:60, 03:74-03:75, 03:80, 03:85, 03:88, 03:90, 03:102-03:103, 03:115	*08:20, 11:17, 11:37:01-11:37:02, 11:41, 11:48, 11:50, 11:52, 11:56, 11:83, 11:107, 11:145, 11:149, 11:170-11:171, 13:03:01-13:03:06, 13:07:01-13:08, 13:12:01-13:13, 13:19, 13:26:01-13:26:02, 13:32-13:33:03, 13:36-13:38, 13:40, 13:47-13:49, 13:53, 13:55, 13:58, 13:60, 13:65, 13:70, 13:72, 13:76, 13:81, 13:84-13:85, 13:88-13:90, 13:94:01-13:95, 13:101, 13:108, 13:115, 13:118, 13:120, 13:122, 13:133-13:135, 13:149, 13:151-13:152, 13:159, 13:164, 13:167, 13:174, 13:180, 13:182, 13:188-13:189, 13:191, 13:194, 14:01:01-14:03:01, 14:05:01-14:09, 14:12:01-14:14, 14:16, 14:18-14:20, 14:22-14:27, 14:29, 14:32:01-14:32:03, 14:34, 14:36-14:41, 14:43-14:45, 14:47-14:49, 14:51, 14:54:01-14:56, 14:58-14:60, 14:62-14:63, 14:67, 14:69-14:70, 14:74-14:75, 14:77-14:78, 14:80-14:92N, 14:94, 14:96-14:106, 14:108-14:119, 14:121-14:125, 14:127:01-14:127:02, 14:129-14:131, 14:133, 14:135-14:137N, 14:139-14:140, 14:142, 14:144, 14:146-14:147, 14:149, 14:151, 14:153-14:159, DRB3*01:14
11⁵	105 bp 180 bp	515 bp	*03:09, 03:23 *03:13:01-03:13:02, 03:38, 03:79, 03:88	*11:116, 11:120, 13:39, 13:54, 13:77-13:79, 13:100, 13:162-13:163, 13:181, 14:48, 14:64, 14:109, 14:115, DRB3*01:14
12	135 bp 190 bp	430 bp	*03:10, 03:110 *03:51, 03:76	*14:82, 14:132 *11:59 ^w , 11:80 ^w , 11:83 ^w , 11:87 ^w , 11:135 ^w , 11:142 ^w , 13:27 ^w , 13:41 ^w , 13:71 ^w , 13:129 ^w , 13:176 ^w
13	165 bp 225 bp	430 bp	*03:47, 03:62 *03:11:01, 03:17, 03:24, 03:27, 03:35, 03:81	*11:136, 14:38:01-14:38:02, 14:127:01-14:127:02
14^{5,6}	105 bp 135 bp 245 bp	515 bp	*03:16 *03:12, 03:110 *03:68N	*09:08
15	155 bp	430 bp	*03:17, 03:37, 03:62, 03:80	*11:39, 11:43, 11:50, 11:115, 11:145, 11:151, 11:168, 11:171, 11:174, 13:118, 13:131, 13:135, 13:150, 14:59, 14:86, 14:96, 14:153-14:154
16⁵	50 bp	430 bp	*03:14-03:15, 03:90	*04:98:01-04:98:02, 11:105
17⁵	100 bp 255 bp	430 bp	*03:93 *03:20	*13:125 *11:06:01-11:06:03, 11:21, 11:47, 11:57, 11:129, 11:174, 13:58, 13:81, 13:163, 14:29, 14:99

101.113-24/04 – including *Taq* polymerase, IFU-01
101.113-24u/04u – without *Taq* polymerase, IFU-02

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Lot No.: 40Y

Lot-specific information

18⁵	120 bp	430 bp	*03:01:01:01-03:01:24, 03:04:01-03:05:03, 03:07- 03:10, 03:12-03:16, 03:18-03:23, 03:25, 03:28-03:30, 03:32-03:34, 03:36-03:37, 03:39-03:40, 03:42-03:52, 03:54-03:59, 03:61-03:73, 03:78-03:79, 03:82-03:83, 03:86-03:87, 03:91-03:94, 03:96, 03:98-03:101, 03:104- 03:114, 03:116-03:118	*11:07, 11:103, 11:105, 11:125, 11:173, 15:25
19	175 bp	515 bp	*03:22	
20⁵	75 bp	430 bp	*03:25, 03:31, 03:75, 03:118	*11:80, 11:135, 11:142
	120 bp 245 bp		*03:28 *03:68N	
21⁵	70 bp	430 bp	*03:21, 03:30, 03:43	*11:10:01-11:10:02, 11:12:01-11:13:02, 11:114 12:05, 12:14-12:15, 13:57, 13:64, 13:83, 13:107, 13:175, 13:195, 14:35, 14:65, 14:72, 14:138 *11:30, 13:67, 13:195
	150 bp		*03:42, 03:87	
22^{5,6}	95 bp 215 bp	430 bp	*03:18 *03:48	
23^{5,6}	65 bp 165 bp	430 bp	*03:09, 03:23 *03:19	
24^{5,8}	65 bp	430 bp	*03:02:01-03:03, 03:27, 03:29, 03:38, 03:53, 03:74, 03:88, 03:90, 03:101-03:103, 03:115	*13:15, 13:26:01-13:26:02, 13:53, 13:85, 13:104, 14:02:01-14:03:02, 14:06:01- 14:06:03, 14:12:01-14:13, 14:18-14:19, 14:24, 14:27, 14:29, 14:46-14:48, 14:51- 14:52, 14:63, 14:67, 14:78, 14:81, 14:85, 14:89, 14:98, 14:106, 14:108-14:109, 14:115, 14:121, 14:135, 14:154, DRB3*02:20
25⁵	80 bp	430 bp	*03:13:01-03:13:02, 03:67N, 03:88	DRB3*01:01:03, DRB3*02:19
	180 bp 230 bp		*03:32 *03:69	
26^{5,7}	115 bp	515 bp	*03:26, 03:33, 03:43- 03:44, 03:71	*11:44, 11:49:01-11:50, 11:76, 11:85, 11:114, 11:127, 13:34, 13:64, 13:106, 13:136, 13:174, 14:41, 14:77, 14:97, 14:110, DRB3*01:14
27^{5,7}	115 bp	515 bp	*03:43, 03:71	*11:76, 11:114, 13:34, 13:64, 13:136, 13:174, 14:41, 14:77, 14:110, DRB3*01:14 *14:150
	150 bp 175 bp 225 bp		*03:53 *03:46 *03:11:01, 03:17, 03:24, 03:27, 03:34, 03:35, 03:51, 03:55, 03:81	*11:53, 11:136
28⁵	75 bp 160 bp 195 bp	430 bp	*03:67N *03:36 *03:45	

101.113-24/04 – including *Taq* polymerase, IFU-01
101.113-24u/04u – without *Taq* polymerase, IFU-02

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Lot No.: 40Y			Lot-specific information	
29	155 bp	430 bp	*03:40-03:41, 03:52, 03:75, 03:105, 03:118	*11:07, 11:103, 11:105, 11:107, 11:125, 11:173
	180 bp		*03:39	
	215 bp		*03:92	*14:76, 14:79
30	175 bp	430 bp	*03:01:01:01-03:07, 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- 03:118	*08:20, 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, 13:171:02-13:174, 13:176-13:178, 13:180- 13:182, 13:184-13:190, 13:192-13:194, 13:196, 14:02:01-14:03:02, 14:05:01- 14:06:03, 14:09, 14:12:01-14:14, 14:17- 14:21, 14:23:01, 14:23:03-14:24, 14:27, 14:29-14:30, 14:33, 14:36-14:37, 14:40- 14:45, 14:47-14:48, 14:51, 14:56, 14:59, 14:63-14:65, 14:67, 14:77-14:78, 14:80- 14:81, 14:83-14:85, 14:89, 14:91, 14:94- 14:96, 14:98, 14:100, 14:102-14:103, 14:106, 14:108-14:109, 14:115-14:116, 14:121, 14:123, 14:127:01-14:127:02, 14:134-14:136, 14:144, 14:154-14:156, 14:159
	205 bp 245 bp	515 bp	*03:45, *03:92 *03:50	*14:76, 14:79
32⁹	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*03 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.

⁴Due to the sharing of sequence motifs within the DR52 group of DRB1 alleles, non-DRB1*03 alleles are amplified by primer mixes 1 to 4, 6 to 18, 20, 21, 24, 26, 27, 29 to 31. In addition, a few DRB3 alleles will be amplified by primer mixes 3, 8 to 11 and 24 to 27.

101.113-24/04 – including *Taq* polymerase, IFU-01
101.113-24u/04u – without *Taq* polymerase, IFU-02

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Lot No.: 40Y

Lot-specific information

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

⁶Primer mixes 14, 22 and 23 may have a tendency of giving rise to primer oligomer formation.

⁷Primer mixes 26 and 27 may have a tendency of unspecific amplification.

⁸Primer mixes 2 and 24 may give rise to a lower yield of specific PCR product than the other DRB1*03 primer mixes.

⁹Primer 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.

‘?’ , nucleotide sequence information not available for the primer matching sequence.

“w” , may be weakly amplified.

101.113-24/04 – including *Taq* polymerase, IFU-01
101.113-24u/04u – without *Taq* polymerase, IFU-02

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Lot No.: **40Y**

Lot-specific information
PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	220	85	155	190	75	105	130	185	135	145	105	135
			190		165						180	190
Length of int. pos. control ¹	515	430	515	430	430	430	430	430	430	430	515	430
5'-primer(s) ²	26(164) 5'-gTA 3'	13(125) 5'-gTC 3'	36(196) 5'-AgA 3'	36(196) 5'-AgA 3'	26(164) 5'-gTA 3'	26(164) 5'-gTA 3'	26(164) 5'-gTA 3'	26(164) 5'-gTT 3'	26(164) 5'-gTA 3'	13(125) 5'-gTC 3'	13(125) 5'-gTC 3'	26(164) 5'-gTA 3'
3'-primer(s) ³	86(344) 5'-CCA 3'	28(171) 5'-CTC 3'	74(308) 5'-CCT 3'	86(344) 5'-CCA 3'	37(197) 5'-Cgg 3'	47(227) 5'-ggA 3'	53(247) 5'-CCg 3'	74(308) 5'-CCC 3'	57(257) 5'-CAT 3'	47(227) 5'-ggT 3'	34(188) 5'-CCC 3'	57(257) 5'-CAg 3'
			86(344) 5'-CAC 3'		66(286) 5'-gAA 3'	47(229) 5'-CCT 3'	58(260) 5'-CCT 3'		57(258) 5'-gCg 3'		60(266) 5'-Agg 3'	57(257) 5'-CAg 3'
												74(308) 5'-CCg 3'
												79(324) 5'-CTC 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	165	105	155	50	100	120	175	75	70	95	65	65
		135			255			120	150	215	165	
		245						245				
Length of int. pos. control ¹	430	515	430	430	430	430	515	430	430	430	430	430
5'-primer(s) ²	13(125) 5'-gTC 3'	26(164) 5'-gTA 3'	13(125) 5'-gTC 3'	74(308) 5'-CCg 3'	13(125) 5'-gTC 3'	47(227) 5'-gTT 3'	31(181) 5'-TCg 3'	26(164) 5'-gTA 3'	10(116) 5'-gCT 3'	26(164) 5'-gTA 3'	26(164) 5'-gTA 3'	28(171) 5'-gAg 3'
						47(227) 5'-ATT 3'			37(197) 5'-gTT 3'			
									37(197) 5'-gTT 3'			
3'-primer(s) ³	51(241) 5'-CTg 3'	47(229) 5'-CCA 3'	51(239) 5'-CCC 3'	77(317) 5'-Agg 3'	32(182) 5'-TAC 3'	73(305) 5'-ggC 3'	77(317) 5'-AgT 3'	36(196) 5'-gTA 3'	47(227) 5'-ggA 3'	44(220) 5'-CCg 3'	34(188) 5'-CCC 3'	36(196) 5'-gTT 3'
	56(254) 5'-CAC 3'	56(256) 5'-gCT 3'	51(241) 5'-CTg 3'		85(341) 5'-CAg 3'			51(241) 5'-CTT 3'		84(340) 5'-AAT 3'	66(286) 5'-gAT 3'	
	74(308) 5'-CCT 3'	57(257) 5'-CAg 3'						93(367) 5'-TCA 3'				
		93(367) 5'-TCA 3'										
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

101.113-24/04 – including *Taq* polymerase, IFU-01
101.113-24u/04u – without *Taq* polymerase, IFU-02

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Lot No.: **40Y**

Lot-specific information

Well No.	25	26	27	28	29	30	31
Length of spec. PCR product	80	115	115	75	155	175	205
	180		150	160	180		245
	230		175	195	215		
			225				
Length of int. pos. control ¹	430	515	515	430	430	430	515
5'-primer(s) ²	10(118) 5'-ACg 3'	13(125) 5'-gTC 3'	13(125) 5'-gTC 3'	21(149) 5'-gAT 3'	15(133) 5'-gTT 3'	13(125) 5'-gTC 3'	5(103) 5'-CAg 3'
	26(166) 5'-ACg 3'			33(186) 5'-AAA 3'	27(167) 5'-CCg 3'		15(133) 5'-gTT 3'
	60(266) 5'-gTC 3'			61(269) 5'-CTA 3'	34(189) 5'-CAA 3'		21(149) 5'-gAT 3'
	61(269) 5'-CTA 3'						
3'-primer(s) ³	73(305) 5'-ggC 3'	37(199) 5'-CAg 3'	37(199) 5'-CAg 3'	73(305) 5'-ggC 3'	73(305) 5'-ggC 3'	58(260) 5'-Cgg 3'	73(305) 5'-ggC 3'
		38(200) 5'-gCg 3'	48(232) 5'-Cgg 3'			58(260) 5'-CAg 3'	
			58(260) 5'-CgC 3'				
			73(305) 5'-ggC 3'				
			75(313) 5'-gTT 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.

101.113-24/04 – including *Taq* polymerase, IFU-01
101.113-24u/04u – without *Taq* polymerase, IFU-02

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Lot No.: 40Y

Lot-specific information

[illegible]

101.113-24/04 – including *Taq* polymerase, IFU-01
101.113-24u/04u – without *Taq* polymerase, IFU-02

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Lot No.: **40Y**

Lot-specific information

CELL LINE VALIDATION SHEET																						
DRB1*03 SSP subtyping ²																						
					Well																	
					17	18	19	20	21	22	23	24	25	26	27	28	29	30	31			
					201432617	201432618	201432619	201432620	201432621	201432622	201432623	201432624	201432625	201432626	201432627	201432628	201432629	201432630	201432631			
IHCW cell line ¹				DRB1																		
1	9001	SA	*01:01	*04:05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
2	9280	LK707	*15:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
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	*13:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
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		*11:01	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-		
18	9257	32367	*09:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
19	9038	BM16	*12:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
20	9059	SLE005	*13:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-		
21	9064	AMALA	*14:02	*14:01	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-		
22	9056	KOSE	*13:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-		
23	9124	IHL	*08:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-		
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	*04:06	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-		
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		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
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	*12:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-		
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		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
48	9303	TER-ND	*01:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

101.113-24/04 – including *Taq* polymerase, IFU-01
101.113-24u/04u – without *Taq* polymerase, IFU-02

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“Instructions for Use” (IFU)

Lot No.: 40Y

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 7, 11 to 17, 19 to 23, 25 to 29 and 31 were available. The specificities of the primers in primer solutions 7, 11 to 17, 21, 26, 27, 29 and 31 were tested by separately adding one additional 5'-primer, respectively one or two additional 3'-primers. In primer solutions 19, 25 and 28 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 20, 22 and 23 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 5 to 7, 11 to 15, 17 and 27 one or several of the 3'-primers were not possible to test. In primer solutions 18, 21, 29 and 31 one of the 5'-primers was not possible to test. In primer solutions 3 and 9 one additional 3'-primer was tested by adding one additional 5'-primer.

101.113-24/04 – including *Taq* polymerase, IFU-01
101.113-24u/04u – without *Taq* polymerase, IFU-02

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Lot-specific information

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Lot No.: **40Y**

Lot-specific information

ADDRESSES:

Manufacturer:

Olerup SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.

Tel: +46-8-717 88 27

Fax: +46-8-717 88 18

E-mail: info-ssp@olerup.com

Web page: <http://www.olerup-ssp.com>

Distributed by:

Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.

Tel: +43-1-710 15 00

Fax: +43-1-710 15 00 10

E-mail: support-at@olerup.com

Web page: <http://www.olerup.com>

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382

Tel: 1-877-OLERUP1

Fax: 610-344-7989

E-mail: info.us@olerup.com

Web page: <http://www.olerup.com>

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.