

101.623-12 – including *Taq* pol., IFU-01
 101.623-12u – without *Taq* pol., IFU-02

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

Lot No.: 61Y

Lot-specific information
Olerup SSP[®] HLA-C*08

Product number:	101.623-12 – including <i>Taq</i> polymerase
	101.623-12u – without <i>Taq</i> polymerase
Lot number:	61Y
Expiry date:	2018-February-01
Number of tests:	12
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. 61Y.

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

**CHANGES COMPARED TO THE PREVIOUS OLERUP SSP[®]
 HLA-C*08 LOT (72V)**

The HLA-C*08 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

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 HLA-C*08 primer set, specificity and interpretation tables have been updated for the HLA-C alleles described since the previous *Olerup SSP[®] HLA-C*08* lot was made (**Lot No. 72V**). The kit design is based on IMGT/HLA database 3.20.0.

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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Lot No.: 61Y

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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
5	-	Added	3'-primers added for the C*08:73 allele.
6	Added	Added	Primer pair added from well 16.
7	-	Added	3'-primer added for the C*08:115 allele.
8	Moved, added	-	5'-primers moved to wells 11, 25 and 28, 5'-primer added for the C*08:112 allele.
11	Added	-	5'-primer added from well 8.
14	Added	-	5'-primers added for the C*08:90 and C*08:95 alleles.
16	Moved	Moved, modified	Primer pairs moved to wells 6 and 26, 3'-primer modified for increased yield, strength of control primer pairs has been optimized.
18	Added	-	5'-primers added for the C*08:92 alleles.
23	Added	-	5'-primers added for the C*08:69, C*08:92, C*08:95 and C*08:74 alleles.
24	Added	-	5'-primers added for the C*08:74 alleles.
25	Added	-	5'-primer added from well 8.
26	Added	Added	Primer pair added from well 16, 3'-primer added for the C*08:107 allele.
28	Added	-	5'-primer added from well 8.
30	Added	-	5'-primer added for the C*08:110 allele.
32	-	-	Updated negative control.

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Lot No.: 61Y

Lot-specific information

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'-primer ¹	164 5'-CAC ^{3'}	340 5'-Agg ^{3'}	440 5'-TTA ^{3'}	45 5'-Tgg ^{3'}	45 5'-Tgg ^{3'}	43 5'-Tgg ^{3'}	36 5'-TAC ^{3'}
							36 5'-TAT ^{3'}
3'-primer ²	231 5'-TgC ^{3'}	2 nd I 5'-AAA ^{3'}	507 5'-TTg ^{3'}	59 5'-CTC ^{3'}	58 5'-ggC ^{3'}	57 5'-CTC ^{3'}	47 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 codonnumbering 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 No.: 61Y

Lot-specific information
PRODUCT DESCRIPTION

HLA-C*08 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the C*08:01 to C*08:119 alleles.

PLATE LAYOUT

Each HLA-C*08 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 PCR plate is marked with ‘HLA-C*08’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘61Y’.

Wells 1 to 31– HLA-C*08 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 between HLA-C alleles non-HLA-C*08 alleles will be amplified by primer mixes 1 to 7, 9, 11 to 28, 30, 31 amplify non-HLA-C*08 alleles. In addition, a few HLA-A and HLA-B alleles will be amplified by primer mixes 1, 3, 5 to 7, 11, 14, 20, 22, 23, 25, 26, 28, 30 and 31.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-C*08 alleles, i.e. **C*08:01 to C*08:119**, recognized by the HLA Nomenclature Committee in April 2015¹ will be amplified by the primers in the HLA-C*08 SSP kit^{2,3}.

The HLA-C*08 kit enables separation of the confirmed HLA-C*08 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 HLA-C*08 alleles is listed below.

The HLA-C*08 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 HLA-C*08 primer set cannot distinguish the following silent mutations: the C*08:01:01-08:01:06 and C*08:01:08-08:01:19 alleles, the C*08:02:01-08:02:05 and C*08:02:08-08:02:12 alleles, the 08:03:01-08:03:03 alleles, the C*08:04:01-08:04:03 alleles, the C*08:08:01-08:08:02 alleles, the 08:15:01-08:15:02 alleles, the C*08:16:01-08:16:02 alleles or the C*08:72:01-08:72:02 alleles.

The following HLA-C*08 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
C*08:01:07, 08:44	28	C*08:36N, 08:78	23
C*08:20, 08:99	30	C*08:55N, 05:64:02	27
C*08:24, 08:88N	9	C*08:69-08:70Q	23
C*08:30, 08:32	19		

¹HLA-C alleles listed on the IMGT/HLA web page 2015-April-17, release 3.20.0, 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 HLA-C*08 primer set cannot separate the 08:15:01-08:15:02 and 08:51 alleles from the C*07:148 and 07:161. These alleles can be distinguished by the HLA-C low resolution kit and/or the HLA-C*07 high resolution kit.

The HLA-C*08 primer set cannot separate the C*08:55N from the C*05:92N allele. These alleles can be distinguished by the HLA-C low resolution kit.

The HLA-C*08 primer set cannot separate the C*08:94 from the C*05:29:01-05:29:02 alleles. These alleles can be distinguished by the HLA-C low resolution kit and/or the HLA-C*05 high resolution kit.

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Lot No.: 61Y

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ALLELE CONFIRMATION STATUS

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

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2015-April-17, release 3.20.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-C*08 homo- and heterozygotes is available upon request.



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SPECIFICITY TABLE

HLA-C*08 SSP subtyping

Specificities and sizes of the PCR products of the 31+1 primer mixes used for HLA-C*08 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-C*08 alleles ³	Other amplified HLA Class I alleles ⁴
1	250 bp	800 bp	*08:01:01-08:01:19, 08:03:01-08:04:03, 08:06, 08:08:01-08:11, 08:13- 08:14, 08:16:01-08:16:02, 08:20-08:22, 08:24, 08:26N, 08:36N, 08:38-08:42, 08:44, 08:46, 08:50, 08:54, 08:56- 08:61, 08:65-08:66, 08:72:01-08:72:02, 08:78- 08:89N, 08:91, 08:93, 08:95- 08:99, 08:101-08:102, 08:104-08:106, 08:109, 08:113, 08:117, 08:119	*01:22, 01:35, 02:03, 02:16:01- 02:16:02, 02:18, 04:04:01-04:04:02, 04:06, 04:13, 04:34, 04:58, 04:122, 04:160, 04:178, 05:11, 05:17, 05:27, 05:68, 05:79, 05:115, 06:04, 06:118, 12:14:01-12:14:02, 12:18:01- 12:18:02, 12:20, 12:83, 14:06, 14:15, 14:53, 15:02:01:01-15:07, 15:09- 15:13, 15:15-15:19, 15:21-15:24, 15:26-15:50, 15:52-15:73, 15:76- 15:83, 15:85-15:101, 15:103-15:113, 16:35, 16:40, 16:48, 17:01:01:01- 17:16, 17:18-17:29, B*58:02
2 ⁵	115 bp	1070 bp	*08:01:01-08:01:19, 08:03:01-08:03:03, 08:06, 08:08:01-08:11, 08:14, 08:16:01-08:16:02, 08:20- 08:22, 08:24, 08:26N, 08:36N, 08:38, 08:40-08:42, 08:44, 08:46, 08:50, 08:56, 08:58-08:61, 08:78-08:89N, 08:91, 08:95-08:99, 08:101- 08:102, 08:105-08:106, 08:109, 08:117, 08:119	*05:79
3 ⁵	115 bp	800 bp	*08:02:01:01-08:02:12, 08:04:01-08:05, 08:07, 08:12-08:13, 08:17-08:19, 08:23, 08:25, 08:27-08:35, 08:37, 08:43, 08:45, 08:48- 08:49, 08:52N-08:55N, 08:62, 08:66-08:71, 08:73- 08:76, 08:90, 08:92-08:94, 08:100, 08:103-08:104, 08:107-08:108, 08:110- 08:116	*03:251, 04:120, 05:01:01:01- 05:01:31, 05:03-05:08, 05:10-05:11, 05:13-05:16, 05:18:01-05:51Q, 05:53-05:61, 05:63-05:67, 05:69- 05:75, 05:77-05:78, 05:80-05:87, 05:89-05:114, 05:116, 06:129, 07:41, B*14:32, B*15:337
4 ⁵	110 bp	1070 bp	*08:03:01-08:03:03, 08:06, 08:14, 08:38, 08:40, 08:61, 08:101, 08:117	*05:79
5	160 bp	1070 bp	*08:05, 08:15:01-08:15:02, 08:21, 08:51, 08:73	*01:13, 02:51, 03:87:01-03:87:02, 04:129, 05:09:01-05:09:03, 05:17, 05:42, 05:46, 05:52, 06:67, 07:101, 07:130, 07:148, 07:161, 12:127, 12:144, 16:27, 17:05, B*15:33, B*15:248
6 ⁵	65 bp 100 bp	800 bp	*08:102 *08:14, 08:80, 08:103	*07:06, 07:18-07:19 *01:02:34, 01:21, 02:42, 04:140,



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Lot No.: 61Y

Lot-specific information

				04:166, 05:98, 06:05, 07:02:09, 12:16, 12:147, 15:63, 15:113, 16:80, B*67:02
	270 bp 310 bp		*08:06 *08:23	*16:33
7⁵	100 bp 150 bp	1070 bp	*08:07, 08:47, 08:104 *08:41, 08:115	*05:23, 05:62, 07:01:48, 07:02:35 *03:87:01, 03:267, 05:27, 05:39, B*15:01:03
	230 bp		*08:87	
8	240 bp	1070 bp	*08:112	
9	140 bp 375 bp 505 bp	1070 bp	*08:88N *08:24, 08:75 *08:10	*02:83 *05:79
10⁵	110 bp 140 bp	800 bp	*08:09 *08:17	
11⁶	225 bp	800 bp	*08:09, 08:11, 08:59, 08:113	*05:04:01-05:04:02, 05:103, 06:129, 07:68, 07:260, 07:302, B*15:337 , B*18:83
	255 bp 280 bp		*08:86 *08:12	*05:16, 05:85, 05:107, 06:129, 07:364, B*14:32
12	165 bp	800 bp	*08:01:01-08:09, 08:11- 08:12, 08:14-08:15:02, 08:17, 08:19-08:24, 08:26N- 08:54, 08:56-08:63, 08:65- 08:93, 08:95-08:117, 08:119	*01:43, 02:87, 03:280, 07:101, 07:148, 07:161, 12:127
13⁵	105 bp	800 bp	*08:02:01:01-08:02:12, 08:05, 08:07, 08:12, 08:17- 08:19, 08:23, 08:25, 08:28, 08:30, 08:32-08:35, 08:37, 08:43, 08:45, 08:47-08:48, 08:52N-08:53, 08:55N, 08:62-08:63, 08:67-08:71, 08:73-08:77, 08:90, 08:92, 08:94, 08:100, 08:103, 08:107-08:108, 08:110- 08:112, 08:114-08:116	*03:251, 04:120, 05:01:01:01- 05:01:31, 05:03-05:07N, 05:10, 05:12-05:16, 05:18:01-05:26, 05:28- 05:51Q, 05:53-05:61, 05:63-05:67, 05:69, 05:71-05:78, 05:80-05:88, 05:90-05:105, 05:107-05:111, 05:113N-05:114, 05:116, 07:41
14	130 bp 160 bp	1070 bp	*08:90 *08:18, 08:95	*03:155, 03:282, 07:413, 12:149 B*35:252
	280 bp		*08:08:01-08:08:02	
15⁶	265 bp	1070 bp	*08:13, 08:16:01-08:16:02, 08:25, 08:94	*05:29:01-05:29:02
16^{5,7}	545 bp	1070 bp	*08:22, 08:56	*01:85, 15:29, 15:87
17	375 bp 430 bp	1070 bp	*08:05, 08:21, 08:25 *08:28	*05:42 ^w , 05:46 *05:25, 05:42
18⁵	80 bp 200 bp	1070 bp	*08:31 *08:26N, 08:92	*05:105
19⁵	110 bp 250 bp	800 bp	*08:27, 08:29-08:31 *08:32	*05:08, 05:89, 05:106, 06:129
20⁵	105 bp	1070 bp	*08:35, 08:43, 08:54	*05:44:01, B*15:298
21⁶	195 bp	1070 bp	*08:01:01-08:01:19, 08:03:01-08:03:03, 08:06,	*01:02:06, 01:04, 01:21, 01:97, 02:02:05, 02:02:13, 02:05:01, 02:06,



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			08:08:01-08:11, 08:14, 08:16:01-08:16:02, 08:20- 08:22, 08:24, 08:26N-08:27, 08:33:02-08:33:03, 08:35- 08:36N, 08:38-08:42, 08:44, 08:46, 08:50, 08:54, 08:56, 08:58-08:61, 08:65-08:66, 08:72:01-08:72:02, 08:78- 08:89N, 08:91, 08:95-08:99, 08:101-08:102, 08:105- 08:106, 08:109, 08:117, 08:119
22⁵	80 bp 155 bp	1070 bp	*08:41, 08:115 *08:33:01
			*05:27, 05:39, B*15:337, B*44:148 *05:18:02-05:18:03, 05:103, 05:107, 05:115, 07:04:01-07:04:10, 07:11- 07:12, 07:63, 07:68, 07:101, 07:139, 07:142, 07:181, 07:199:01- 07:199:02, 07:260, 07:272, 07:302, 07:323-07:324, 07:328-07:329N, 07:336, 07:338, 07:354-07:355, 07:357-07:358, 07:361, 07:364- 07:365, 07:378, 07:394-07:395, 07:403, 07:406, B*14:32, B*18:83, B*44:148
23⁵	90 bp 140 bp 205 bp 240 bp	1070 bp	*08:36N, 08:69 *08:92, 08:95 *08:70Q *08:78
			*03:174, 07:229, 07:387, 12:131, 14:39, 16:42, 16:56, B*07:243, B*08:98, B*35:202 *03:155, B*35:252 *03:236, 04:106
24⁵	105 bp 150 bp	1070 bp	*08:56, 08:89N *08:37, 08:53, 08:74
			*04:179 *02:14:01-02:14:02, 04:42:01- 04:42:02, 05:43, 06:05, 07:02:09, 12:16, 12:147, 15:23, 15:63, 16:21, 16:80
25⁵	115 bp 225 bp 265 bp	1070 bp	*08:39 *08:09, 08:11, 08:59, 08:113 *08:62, 08:82
			*05:12, A*29:10, B*18:83, B*44:148 *05:04:01-05:04:02, 05:103, 06:129, 07:68, 07:260, 07:302, B*15:337, B*18:83 *03:251, 05:05, A*02:425, A*02:519, A*29:10, A*68:69, B*14:32, B*15:337, B*18:83, B*44:148



101.623-12 – including *Taq pol.*, IFU-01
 101.623-12u – without *Taq pol.*, IFU-02

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 “Instructions for Use” (IFU)

Lot No.: 61Y

Lot-specific information

	290 bp		*08:34	
26⁵	100 bp	1070 bp	*08:14, 08:80, 08:103	*01:02:34, 01:21, 02:42, 04:140, 04:166, 05:98, 06:05, 07:02:09, 12:16, 12:147, 15:63, 15:113, 16:80, B*67:02
	125 bp		*08:42	
27⁵	200 bp		*08:107	
	125 bp	1070 bp	*08:19, 08:101	*05:64:01-05:64:02
28	265 bp		*08:55N	*05:92N
	155 bp	1070 bp	*08:53	
29	185 bp		*08:01:07, 08:02:07, 08:33:02	*03:251, 04:120
	290 bp		*08:34, 08:44, 08:61, 08:82	*03:251, 05:10, B*44:148
30⁵	205 bp	1070 bp	*08:38, 08:52N	
30⁵	65 bp	1070 bp	*08:99	
	270 bp		*08:110	
31	405 bp		*08:28	*05:25, 05:42, 07:01:48, 07:02:35, 07:41, A*01:01:06
	470 bp		*08:20, 08:40, 03:171, 03:211:01, 04:144, 05:93, 06:73, 12:109	
32⁸	500 bp	1070 bp	*08:02:06, 08:19, 08:62	*04:129, 05:01:20, 05:64:01, A*32:72
32⁸	-	-	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 HLA-C*08 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 inherit feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 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 HLA Class I alleles 1st and/or 4th 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 between HLA-C alleles non-HLA-C*08 alleles will be amplified by primer mixes 1 to 7, 9, 11 to 28, 30, 31 amplify non-HLA-C*08 alleles. In addition, a few HLA-A and HLA-B alleles will be amplified by primer mixes 1, 3, 5 to 7, 11, 14, 20, 22, 23, 25, 26, 28, 30 and 31.

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

⁶Primer mixes 11, 15 and 21 may have tendencies of unspecific amplifications.



**101.623-12 – including *Taq pol.*, IFU-01
101.623-12u – without *Taq pol.*, IFU-02**

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“Instructions for Use” (IFU)

Lot No.: 61Y

Lot-specific information

⁷Primer mix 16 may give rise to a lower yield of HLA-specific PCR product than the other HLA-C*08 primer mixes.

⁸Primer 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’, might be weakly amplified.



101.623-12 – including *Taq pol.*, IFU-01
 101.623-12u – without *Taq pol.*, IFU-02

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 "Instructions for Use" (IFU)

Lot No.: 61Y

Lot-specific information
PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12	
Length of spec. PCR product	250	115	115	110	160	65	100	240	140	110	225	165	
						100	150		375	140	255		
						270	230		505		280		
						310							
Length of int. pos. control ¹	800	1070	800	1070	1070	800	1070	1070	1070	800	800	800	
5'-primer(s) ²	2 nd I 5'-CCA 3' 5'-TAC 3' 5'-TgA 3' 5'-TAC 3'	527 527 485 1018 5'-CAA 3'	527 176 2 nd I 5'-CCA 3' 5'-CTA 3'	142 453 658 5'-AgC 3'	404 312 176 5'-CCA 3'	361 361 429 736 757 5'-gCA 3'	176 312 419 634 5'-gTC 3'	361 361 419 634 5'-gCA 3'	176 312 419 634 5'-gCA 3'	361 361 419 634 5'-CAA 3'	176 312 419 634 5'-CAA 3'	176 312 419 634 5'-gCA 3'	
3'-primer(s) ³	539 5'-TCA 3'	601 5'-CTT 3'	601 5'-CTT 3'	595 5'-CCT 3'	289 5'-AgC 3'	201 5'-CTT 3'	512 5'-CCA 3'	601 5'-CTT 3'	526 5'-Cgt 3'	277 5'-gCA 3'	601 5'-CTT 3'	302 5'-ggC 3'	
					289 5'-AgC 3'	559 5'-CgC 3'	560 5'-ACA 3'		956 5'-CAG 3'	598 5'-CTC 3'	846 5'-CAC 3'		
						299 5'-TCT 3'	599 5'-TCC 3'	846 5'-CAC 3'					
						601 5'-CTC 3'	1043 5'-CAA 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	105	130	265	545	375	80	110	105	195	80	90	105
					160		430	200			155	140
					280			250				150
Length of int. pos. control ¹	800	1070	1070	1070	1070	800	1070	1070	1070	1070	1070	1070
5'-primer(s) ²	539 5'-gCg 3'	173 5'-CgC 3'	1 st I 5'-CgA 3'	972 5'-CTA 3'	1 st I 5'-CgA 3'	441 5'-TAg 3'	391 5'-ACT 3'	527 5'-TgA 3'	2 nd I 5'-CCA 3'	486 5'-ACC 3'	85 5'-CCg 3'	85 5'-CCg 3'
						194 5'-CgT 3'		443 5'-CAA 3'	520 5'-CgC 3'		560 5'-CCT 3'	194 5'-CgT 3'
											246 5'-CAG 3'	88 5'-Tgg 3'
						215 5'-gCA 3'		560 5'-CgA 3'	539 5'-gTg 3'			97 5'-TCg 3'
						363 5'-AgC 3'					257 5'-CCA 3'	134 5'-CCg 3'
											374 5'-CTA 3'	527 5'-TAC 3'
											443 5'-CAA 3'	
											3 rd I 5'-Cgg 3'	
3'-primer(s) ³	601 5'-CTT 3'	302 5'-ggC 3'	175 5'-CCg 3'	1034 5'-AgT 3'	289 5'-AgC 3'	601 5'-CTT 3'	601 5'-CTT 3'	584 5'-ggC 3'	485 5'-CCg 3'	601 5'-CTT 3'	302 5'-ggC 3'	201 5'-CTT 3'
					601 5'-CTT 3'	175 5'-CCT 3'		341 5'-CgT 3'		595 5'-CCT 3'		538 5'-CCg 3'
												587 5'-TCT 3'
											787 5'-TCT 3'	
Well No.	13	14	15	16	17	18	19	20	21	22	23	24



101.623-12 – including *Taq pol.*, IFU-01
 101.623-12u – without *Taq pol.*, IFU-02

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 “Instructions for Use” (IFU)

Lot No.: 61Y

Lot-specific information

Well No.	25	26	27	28	29	30	31
Length of spec.	115	100	125	155	205	65	500
PCR product	225	125	265	185		270	
	265	200		290		405	
	290					470	
Length of int.	1070	1070	1070	1070	1070	1070	1070
pos. control ¹							
5'-primer(s) ²	351 5'-CAA 3' 379 5'-ACg 3' 419 5'-gTC 3' 527 5'-TgT 3'	142 5'-TCT 3' 2 nd I 5'-CCA 3' 368 5'-gTT 3' 351 5'-CAA 3' 355 5'-TCA 3' 459 5'-gAT 3'	1 st I 5'-CgA 3' 368 5'-gTT 3' 351 5'-g.T 3' 851 5'-CCA 3' 851 5'-CCT 3' 934 5'-TCA 3'	88 5'-Tgg 3' 351 5'-gTC 3' 679 5'-ggA 3' 652 5'-gGA 3' 341 5'-gCA 3'	368 5'-gTC 3' 679 5'-ggA 3' 652 5'-gGA 3' 341 5'-gCA 3'	341 5'-ggA 3' 652 5'-gGA 3' 341 5'-gCA 3'	176 5'-gCA 3'
3'-primer(s) ³	601 5'-CTT 3' 412 5'-CTT 3'	201 5'-CTT 3' 453 5'-CTA 3' 601 5'-CTT 3'	175 5'-CTA 3' 453 5'-CTA 3' 601 5'-CTT 3'	201 5'-CTT 3' 846 5'-CAC 3' 956 5'-CAg 3'	526 5'-CgT 3' 846 5'-CAC 3' 956 5'-CAg 3'	453 5'-TCA 3' 956 5'-CAg 3'	387 5'-TCC 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 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 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.



**101.623-12 – including *Taq* pol., IFU-01
101.623-12u – without *Taq* pol., IFU-02**

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“Instructions for Use” (IFU)

Lot No.: 61Y

Lot-specific information



**101.623-12 – including *Taq* pol., IFU-01
101.623-12u – without *Taq* pol., IFU-02**

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“Instructions for Use” (IFU)

Lot No.: 61Y

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.



101.623-12 – including *Taq pol.*, IFU-01
101.623-12u – without *Taq pol.*, IFU-02

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“Instructions for Use” (IFU)

Lot No.: 61Y**Lot-specific information**

²The C*07:18 allele is amplified by primer mix 6 in the 9285 (WT49) 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.

No DNAs carrying the alleles to be amplified by primer solutions 5, 7 to 11, 14, 15, 17 to 19, 23 to 31 were available.

The specificity of the primers in primer solutions 5, 7, 9, 11, 14, 15, 17 to 19, 23 to 25 and 27 to 31 were tested by adding additional 5'-primers respectively 3'-primers. In primer solution 10 it was only possible to test the 5'-primer, the 3'-primer was not possible to test.

In primer solution 8 it was only possible to test the 3'-primers, the 5'-primers was not possible to test.

In primer solution 7, 9, 11, 14, 18, 19, 23, 24, 25 and 28 to 30, one, two or three 5'-primers were not possible to test. In primer solutions 6, 24, 26 and 27, one or two 3'-primers were not possible to test.

Additional primers in primer solutions 6, 20 and 22 were tested by separately adding either 5'-primers and/or one or two 3'-primers.



**101.623-12 – including *Taq* pol., IFU-01
101.623-12u – without *Taq* pol., IFU-02**

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“Instructions for Use” (IFU)

Lot No.: 61Y

Lot-specific information

101.623-12 – including *Taq pol.*, IFU-01
101.623-12u – without *Taq pol.*, IFU-02

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

Lot No.: 61Y

Lot-specific information



101.623-12 – including *Taq pol.*, IFU-01
101.623-12u – without *Taq pol.*, IFU-02

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

Lot No.: 61Y

Lot-specific information



101.623-12 – including *Taq pol.*, IFU-01
101.623-12u – without *Taq pol.*, IFU-02

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“Instructions for Use” (IFU)

Lot No.: 61Y

Lot-specific information

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