

Lot No.: **1K6**

Lot-specific information

Olerup SSP[®] HLA-C*16

Product number:	101.627-12 – including <i>Taq</i> polymerase 101.627-12u – without <i>Taq</i> polymerase
Lot number:	1K6
Expiry date:	2023-09-01
Number of tests:	12
Number of wells per test:	23+1
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

This Product Description is only valid for Lot No. 1K6.

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*16 LOT (0H4)

- The product documentation has been updated for new alleles of IMGT 3.36.0
- The kit resolution focuses on common and well documented (CWD) alleles¹.

The HLA-C*16 primer set, specificity and interpretation tables have been updated for the HLA-C alleles described since the previous *Olerup SSP[®]* HLA-C*16 lot was made (**Lot No. 0H4**).

¹S. J. Mack, P. Cano, J. A. Hollenbach et al.
Common and well-documented HLA alleles: 2012 update to the CWD catalogue. *Tissue Antigens*, 2013, 81, 194–203

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

Well	5'-primer	3'-primer	rationale
2	Exchanged	Added	5'-primer exchanged for improved yield. 3'-primer added for the C*16:02:15 allele.

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Well **24** contains Negative Control primer pairs, that will amplify a majority 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 products generated by the positive control primer pair are 200 and 430 base pairs.

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

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

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

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PRODUCT DESCRIPTION

HLA-C*16 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the C*16:01 to C*16:152 alleles.

PLATE LAYOUT

Each HLA-C*16 test consists of 24 PCR reactions in a 24 well cut PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	NC

The 24 well PCR plate is marked with 'HLA-C*16' in silver/gray ink.

Well No. 1 is marked with the Lot No. '1K6'.

Wells 1 to 23 – HLA-C*16 high resolution primers.

Well 24 – 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 24 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*16 alleles will be amplified by some primer mixes. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-C*16 alleles, i.e. **C*16:01 to C*16:152**, recognized by the HLA Nomenclature Committee in April 2019^{1,2} will be amplified by the primers in the HLA-C*16 SSP kit³.

The HLA-C*16 kit enables separation of the confirmed HLA-C*16 alleles as listed in the IMGT/HLA database 3.25.0. 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*16 alleles is listed below.

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The HLA-C*16 kit also enables identification of many null and alternatively expressed alleles.

The following HLA-C*16 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
C*16:15:01-16:15:02, 16:20	11	C*16:27, 16:32	20
C*16:16Q, 16:17	12	C*16:28, 16:31, 16:50	19
C*16:24, 16:58	22	C*16:30N, 16:56	23

¹HLA-C alleles listed on the IMGT/HLA web page 2019-April-17, 3.36.0, 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*16 primer set cannot separate the 16:04:01:01-16:04:01:02, 16:04:04-16:04:05, 16:33, 16:66, 16:78, 16:82, 16:109, 16:124, 16:149-16:150 and the C*12:176 alleles. These alleles can be distinguished by the HLA-C low resolution kit and/or the HLA-C*12 high resolution kit.

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ALLELE CONFIRMATION STATUS

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

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2016-July-14, release 3.25.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-C*16 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

HLA-C*16 SSP subtyping

Specificities and sizes of the PCR products of the 23+1 primer mixes used for HLA-C*16 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-C*16 alleles ³	Other amplified HLA Class I alleles
1	210 bp	800 bp	*16:01:01:01-16:02:17, 16:04:01:01-16:04:01:02, 16:04:03-16:04:05, 16:08-16:21, 16:23-16:34, 16:36-16:39:02, 16:41-16:42, 16:44-16:47, 16:49-16:52, 16:54-16:67, 16:69-16:87, 16:89N-16:109, 16:111-16:112, 16:114-16:116, 16:118-16:152	*06:31, 12:176
2	445 bp	1070 bp	*16:02:01-16:02:17, 16:09, 16:12, 16:19, 16:25, 16:46-16:48, 16:57, 16:60, 16:63, 16:69-16:70, 16:74, 16:77N, 16:84, 16:88-16:91, 16:99, 16:101-16:104, 16:107-16:108, 16:115, 16:120, 16:123N, 16:132N-16:133, 16:136, 16:140, 16:143-16:145	*01:14, 01:59, 01:118, 03:07:01:01-03:07:02, 03:15, 03:45, 03:130, 03:140, 03:163, 03:243, 03:268, 03:297, 03:461, 04:01:01:01-04:01:01:29, 04:01:01:31-04:01:28, 04:01:30-04:01:76, 04:01:78-04:01:113, 04:03:01:01-04:10, 04:12-04:20, 04:23-04:28, 04:30-04:35, 04:37-04:54:02, 04:56-04:171, 04:173N-04:213, 04:215N-04:225N, 04:227-04:248, 04:250-04:298, 04:300N-04:375, 05:01:01:01-05:01:45, 05:03-05:128N, 05:130-05:215, 06:02:01:01-06:02:01:17, 06:02:03-06:02:11, 06:02:13-06:02:64, 06:02:66-06:10, 06:12-06:16N, 06:18-06:51, 06:53:01-06:121, 06:123, 06:125-06:146, 06:148-06:216, 06:218-06:235, 06:237-06:243, 06:245-06:247, 06:249-06:251, 06:253-06:263N, 08:10, 12:04:01-12:05, 12:09, 12:21, 12:33, 12:41, 12:54, 12:60, 12:146, 12:188, 14:04, 14:12, 14:49, 14:64, 14:77, 14:108, 17:02, 17:06 ² -17:16:01 ² , 17:18 ² -17:20 ² , 17:23 ² -17:29 ² , 17:31 ² -17:36 ² , 17:45 ²
3 ⁵	220 bp	800 bp	*16:04:01:01-16:04:01:02, 16:04:03-16:04:05, 16:29, 16:33, 16:42, 16:55, 16:61,	*01:04, 01:09, 02:05:01-02:05:03, 02:17, 04:360, 06:02:01:01-06:02:01:17, 06:02:03-06:02:15, 06:02:17-06:02:42, 06:02:44-06:03:02, 06:07-06:13, 06:15-06:34:02, 06:36-06:39, 06:41-06:71, 06:73-06:78, 06:80, 06:82-06:100, 06:102:01-06:117, 06:119-06:122,

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			16:66, 16:78, 16:82, 16:91, 16:109, 16:124, 16:149-16:150	06:124-06:126, 06:128N-06:135, 06:137-06:142, 06:145-06:152N, 06:154-06:196, 06:198-06:202, 06:205-06:263N, 12:03:01:01-12:03:46, 12:03:48-12:07, 12:11-12:13:01:02, 12:15, 12:23, 12:25-12:26, 12:28-12:29, 12:31-12:35, 12:37-12:39N, 12:42Q-12:43, 12:45-12:48, 12:50-12:55, 12:57:01-12:63, 12:65-12:66, 12:70-12:71, 12:75-12:79, 12:81-12:82, 12:87- 12:95, 12:97-12:102, 12:107-12:111, 12:113, 12:115-12:116, 12:119-12:122, 12:125, 12:129, 12:131, 12:133, 12:135, 12:138-12:141, 12:143- 12:144, 12:147, 12:149-12:150, 12:152, 12:154, 12:156-12:160, 12:163, 12:165, 12:167, 12:170- 12:174, 12:176, 12:178, 12:180, 12:182, 12:184- 12:187, 12:189-12:192, 12:194-12:195:03, 12:197, 12:199, 12:201-12:203, 12:205-12:206, 12:209-12:211, 12:213, 12:215-12:216, 12:218, 12:220, 12:223, 12:225, 12:227, 12:229-12:230, 12:232N, 12:235, 12:237-12:238, 12:242, 12:244-12:246, 12:248-12:249, 12:253-12:254, 12:256-12:257, 12:259-12:260, 12:262, 12:264- 12:267, 12:269, 12:271-12:274N, 12:276- 12:278, 12:282, 14:16, B*15:510
4	140 bp	800 bp	*16:01:01:01- 16:01:01:06, 16:01:03- 16:01:15, 16:01:17- 16:01:25, 16:01:27- 16:01:32, 16:04:01:01- 16:04:01:02, 16:04:03- 16:04:05, 16:06- 16:08, 16:10- 16:11, 16:13- 16:18, 16:20- 16:24, 16:26- 16:36, 16:37 ^w , 16:38-16:45, 16:49-16:56, 16:58-16:59, 16:61-16:62, 16:64-16:68, 16:71 ^w , 16:72- 16:73, 16:75- 16:76, 16:78- 16:83, 16:86- 16:87, 16:92- 16:93, 16:95- 16:98, 16:100, 16:105, 16:109- 16:114, 16:116- 16:119, 16:122,	*01:02:34, 01:21, 02:12 ^w , 02:27:01-02:27:02, 02:87, 02:115, 02:126 ^w , 02:131, 03:04:25, 04:11, 04:29, 04:36, 04:55, 04:172, 04:214 ^w , 07:01:74, 07:02:09, 07:583, 07:723, 08:01:01:01-08:02:10, 08:02:12-08:09, 08:11-08:63, 08:65-08:94, 08:95 ^w , 08:96-08:188, 12:02:01-12:02:10, 12:02:12-12:02:22, 12:02:23 ^w , 12:02:24- 12:02:27, 12:02:28 ^w , 12:03:01:01-12:03:03, 12:03:05-12:03:08, 12:03:10-12:03:23, 12:03:24 ^w , 12:03:25-12:03:33, 12:03:35- 12:03:56, 12:06-12:08, 12:10:01-12:16:01, 12:17-12:20, 12:22-12:26, 12:28-12:32, 12:34- 12:40, 12:42Q-12:53, 12:55-12:59, 12:61-12:71, 12:72 ^w , 12:73-12:122, 12:124-12:134, 12:135 ^w , 12:136-12:145, 12:147-12:153, 12:154 ^w , 12:155Q-12:187, 12:189-12:194, 12:195:02, 12:196-12:282, 14:02:03, 14:03:01, 14:08, 14:10, 14:22, 14:35N, 14:38, 14:41, 14:53- 14:54, 14:61, 14:70, 14:79, 14:86, 14:90, 15:07, 15:21 ^w , 15:25, 15:116 ^w , 15:144, B*08:01:36 , B*15:01:50, B*15:436, B*18:03:02, B*35:08:02 , B*35:08:05, B*67:02

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			16:124-16:125, 16:127-16:131, 16:134-16:135, 16:137-16:139, 16:141-16:142, 16:146-16:152	
5	160 bp	800 bp	*16:01:01:01- 16:02:17, 16:06- 16:09, 16:11- 16:28, 16:30N- 16:32, 16:34, 16:36-16:39:02, 16:41, 16:43- 16:44, 16:46- 16:47, 16:49- 16:52, 16:54, 16:56-16:60, 16:62-16:65, 16:69-16:77N, 16:79-16:81, 16:83-16:87, 16:89N-16:90, 16:92-16:102, 16:104-16:108, 16:111-16:123N, 16:125-16:139, 16:141-16:148, 16:151-16:152	*07:53, 07:216, 14:90, A*24:174
6⁴	125 bp	800 bp	*16:11, 16:39:01- 16:39:02	*02:21, 15:191
	160 bp		*16:10	A*24:106
	210 bp		*16:06, 16:115	*07:216
7⁴	100 bp	1070 bp	*16:09	*02:34
	170 bp		*16:45	*04:14, 04:68, 05:112, A*24:96, A*24:146
	210 bp		*16:07:01	
8	130 bp	1070 bp	*16:08, 16:38	*08:96, 15:114
	160 bp		*16:89N	*05:169N
	190 bp		*16:123N	
	435 bp		*16:53, 16:68, 16:88	
9^{4,6}	85 bp	1070 bp	*16:12	*02:79, 04:69
	140 bp		*16:52	*06:133
	205 bp		*16:35, 16:48	*01:160, 06:118, 07:31:01-07:31:02, 07:177, 07:514, 14:15, 14:87
10	215 bp	800 bp	*16:19	*03:420, 04:101, 05:117, 07:114, 07:557, 08:135
	350 bp		*16:13, 16:61	*02:159, 02:161, 05:81, 06:87, 07:24, 07:218, 12:45, 12:166, 14:65
11	170 bp	1070 bp	*16:20	*15:75, A*24:73, A*24:157, B*07:66, B*51:55
	540 bp		*16:15:01- 16:15:02, 16:25, 16:64	*04:14, 04:68, 07:53, 07:216
12⁴	100 bp	1070 bp	*16:17, 16:67	*01:27
	210 bp		*16:22	

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	245 bp		*16:16Q	
13	130 bp	1070 bp	*16:14	*06:32, 12:40
	190 bp		*16:123N	
	240 bp		*16:77N	*02:121N, 06:171:01:01N-06:171:01:02N, 07:164N, 07:451N
14⁶	210 bp	1070 bp	*16:18, 16:23, 16:104	*14:90
15⁴	85 bp	1070 bp	*16:04:03	
	120 bp		*16:39:01-16:39:02	*15:191
	145 bp		*16:21, 16:80	*02:14:01-02:14:02, 02:107, 02:164, 04:42:01-04:42:02, 04:220, 05:43, 06:05, 07:01:74, 07:02:09, 08:37, 12:16:01, 12:147, 12:195:02, 12:217, 15:23:01-15:23:02, 15:63, 15:138, 15:158
16	165 bp	1070 bp	*16:90	*02:83, 08:24
	375 bp		*16:06-16:07:02, 16:117	*01:05, 01:21, 01:36, 01:55, 01:79:01-01:79:02, 01:120, 02:02:01-02:02:03, 02:02:06-02:02:08, 02:02:10-02:02:30, 02:02:32-02:02:48, 02:02:50-02:04, 02:06:01-02:16:02, 02:18-02:36:02, 02:38:01N-02:40:02, 02:42-02:56, 02:58-02:61, 02:63-02:73, 02:75-02:80, 02:82-02:122, 02:124-02:169N, 03:05, 03:13:01-01-03:13:02, 03:25, 03:27, 03:35:01-03:35:02:02, 03:135, 03:167, 03:178, 03:198, 03:267, 03:292, 03:296:01-03:296:02, 03:335, 03:386, 03:407, 04:01:01-01-04:01:01:29, 04:01:01:31-04:01:23, 04:01:25-04:01:113, 04:03:01:01-04:20, 04:23-04:36, 04:38-04:39, 04:41-04:79, 04:81-04:99, 04:101-04:109, 04:111-04:116, 04:118-04:177, 04:179-04:223:02, 04:225N-04:229, 04:231, 04:233N-04:241, 04:243-04:262, 04:264-04:341, 04:343-04:359, 04:361-04:369N, 04:371N-04:375, 05:01:01:01-05:01:20, 05:01:22-05:01:45, 05:03-05:06, 05:08-05:09:03, 05:11-05:15, 05:17-05:30, 05:32-05:84, 05:86-05:95, 05:97-05:103:02, 05:105-05:106:02, 05:108-05:147, 05:149-05:151, 05:153N-05:174, 05:176-05:213N, 05:215, 06:101, 06:127:01-01-06:127:02, 06:136, 06:144, 07:01:01:01-07:01:10, 07:01:12-07:01:27, 07:01:29-07:02:97, 07:02:99-07:03, 07:05-07:06:01:02, 07:07-07:09, 07:13-07:30, 07:32N-07:33N, 07:35-07:42, 07:44, 07:46-07:62, 07:64-07:100, 07:102-07:138, 07:140-07:141:02, 07:143-07:176, 07:178-07:180, 07:182-07:183, 07:185-07:194, 07:197-07:271, 07:273-07:294, 07:296-07:301, 07:303-07:322, 07:325-07:327, 07:330:01-07:331, 07:333-07:335, 07:337, 07:339-07:345, 07:347N-07:353, 07:356, 07:359-07:360, 07:362-07:363, 07:368:01-07:377, 07:379-07:393N, 07:396-07:402, 07:404-07:405, 07:407-07:419, 07:421-07:425, 07:427, 07:429-07:443, 07:445-07:446, 07:448-07:458, 07:460-07:462, 07:464-07:465, 07:468-07:479,

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				07:481-07:484N, 07:486, 07:488-07:500, 07:502-07:510, 07:512-07:513Q, 07:515-07:522, 07:524-07:533, 07:536-07:551N, 07:553-07:561, 07:564-07:567, 07:570-07:584, 07:588, 07:590-07:599, 07:601-07:608, 07:610, 07:612-07:621, 07:623-07:625, 07:627-07:650:02, 07:652-07:654, 07:657-07:663Q, 07:665-07:671, 07:673, 07:675N-07:692, 07:694-07:697Q, 07:699-07:725, 07:727, 07:729-07:730, 07:732-07:741, 07:743N, 07:745N, 07:748-07:750N, 07:752N-07:757, 07:759-07:775, 07:777-07:779, 08:01:01-08:01:10, 08:01:12-08:11, 08:13-08:33:03, 08:35-08:43, 08:45-08:60, 08:62:01-08:63, 08:65-08:81, 08:83-08:125, 08:127N-08:170, 08:172-08:179, 08:181N-08:188, 12:02:01-12:02:11, 12:02:13-12:02:29, 12:08, 12:10:01-12:10:02, 12:14:01-12:14:02, 12:16:01-12:18:02, 12:21-12:22, 12:27, 12:30, 12:36, 12:40-12:41, 12:49, 12:56, 12:64, 12:67-12:69, 12:72-12:74, 12:80N, 12:83-12:86, 12:96, 12:103-12:106, 12:112, 12:114, 12:117-12:118, 12:123-12:124, 12:126-12:128, 12:130, 12:132, 12:136-12:137, 12:142, 12:145-12:146, 12:148N, 12:151, 12:155Q, 12:161-12:162, 12:164, 12:166, 12:168-12:169, 12:177, 12:179, 12:181, 12:188, 12:193, 12:196, 12:198, 12:204, 12:207-12:208, 12:212, 12:214, 12:217, 12:219N, 12:221-12:222, 12:224, 12:226, 12:228, 12:231, 12:233-12:234, 12:236N, 12:239-12:241, 12:243, 12:247, 12:250, 12:252, 12:255, 12:261, 12:263, 12:268, 12:275, 12:279-12:281, 14:09, 14:28:02, 15:22, 15:65, 15:72, 15:190, 17:16:01-17:16:02, 18:01:01-18:12
17	180 bp	1070 bp	*16:26, 16:46, 16:55, 16:64	*02:49, 02:75, 02:115, 04:01:01:01-04:01:01:29, 04:01:01:31-04:01:09, 04:01:11-04:01:22, 04:01:24-04:01:73, 04:01:74 ^w , 04:01:75-04:01:113, 04:03:01:01-04:07:01, 04:08-04:10, 04:12-04:20, 04:23-04:26, 04:28-04:32, 04:34-04:51, 04:53-04:54:02, 04:56-04:106, 04:108-04:115N, 04:117-04:129, 04:131-04:166:01, 04:167-04:168, 04:170N-04:171, 04:173N-04:230, 04:232-04:282, 04:284-04:375, 05:25, 05:42, 06:05, 06:76:02, 07:01:74, 07:02:09, 07:583, 08:28, 08:137, 08:168, 12:28, 12:132, 12:135, 12:146, 15:25, 15:62, 15:169
	235 bp		*16:97	
18⁴	120 bp 255 bp	1070 bp	*16:38 *16:26, 16:46, 16:55, 16:64	*08:96, 15:114 *01:23, 01:58, 02:49, 02:75, 02:115, 04:01:72, 04:03:01:01-04:03:05, 04:06:01, 04:42:02, 04:80, 04:140, 04:147, 04:160, 04:171, 04:220, 04:256, 04:286, 04:294, 04:299, 04:335, 04:337, 04:351:02, 04:357, 04:363, 05:25, 05:42, 06:02:01:01-06:02:01:17, 06:02:03-06:02:09, 06:02:11-06:25, 06:27-06:29, 06:31-06:52,

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				06:54-06:124, 06:126-06:131, 06:133-06:168, 06:170-06:197, 06:199-06:203, 06:205-06:209, 06:211N-06:216, 06:218-06:263N, 07:01:01:01-07:01:22, 07:01:24-07:02:10, 07:02:12-07:02:106, 07:04:01:01-07:04:04, 07:04:06-07:06:02, 07:08-07:15, 07:17:01-07:19, 07:20:02-07:33N, 07:35, 07:37-07:50, 07:52-07:55N, 07:57-07:58, 07:61N-07:63, 07:65-07:73:01, 07:74-07:78:02, 07:80-07:87, 07:89-07:95, 07:96:02-07:108:02, 07:110-07:126, 07:128-07:172:01, 07:173-07:176, 07:178-07:180, 07:182-07:226, 07:228-07:262, 07:264N-07:294, 07:296-07:314:02, 07:315-07:326, 07:329N-07:354, 07:356-07:366, 07:368:01-07:377, 07:379-07:389, 07:391-07:401, 07:403-07:437N, 07:439-07:441:01, 07:442-07:560, 07:562-07:577, 07:579, 07:581-07:597, 07:599-07:667, 07:669-07:722, 07:724-07:755, 07:757-07:779, 08:28, 08:137, 08:168, 12:28, 12:132, 12:135, 12:146, 15:25, 15:62, 15:169, 17:11, 18:01:01-18:12
19⁴	100 bp 240 bp	800 bp	*16:28, 16:67 *16:29, 16:31, 16:50	*01:108, 06:90 *01:10, 02:05:01-02:05:03, 02:17, 06:08, 06:22, 12:119, 14:25, 17:21, B*07:239, B*14:46, B*14:52, B*40:243
20⁴	95 bp 145 bp 210 bp	1070 bp	*16:27 *16:32 *16:23, 16:104	*05:112 *14:90
21	445 bp 595 bp	1070 bp	*16:40, 16:53, 16:110, 16:113 *16:49	
22^{4,7}	85 bp 210 bp	800 bp	*16:58 *16:24	*03:108, 03:150, 04:293, 07:25, 07:404, 15:136
23^{4,6}	95 bp 170 bp	1070 bp	*16:42, 16:56 *16:30N	*04:273, 05:56, 08:69, 12:131
24⁸	-	-	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*16 high resolution SSP typings.

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 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

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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.

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

⁵Primer mix 3 may give rise to a lower yield of HLA-specific PCR product than the other C*16 primer mixes.

⁶Primer mixes 9, 14 and 23 may have tendencies of unspecific amplifications.

⁷Primer mix 22 has a tendency to giving rise to primer oligomer formation.

⁸Primer mix 24 contains a negative control, which will amplify a majority 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 products generated by the HGH positive control primer pair are 200 and 430 base pairs.

Abbreviations

'w' might be weakly amplified.

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

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	210	445	220	140	160	125	100	130	85	215	170	100
						160	170	160	140	350	540	210
						210	210	190	205			245
								435				
Length of int. pos. control ¹	800	1070	800	800	800	800	1070	1070	1070	800	1070	1070
5'-primer(s) ²	360	28	361	201	419	113	244	126	256	385	289	361
	5'-CAg 3'	5'-TCA 3'	5'-AgT 3'	5'-CCA 3'	5'-gTC 3'	5'-CCA 3'	5'-CgC 3'	5'-ggA 3'	5'-ACg 3'	5'-ggT 3'	5'-Agg 3'	5'-AgT 3'
	361					124	369	539	361	523	409	
	5'-AgT 3'					5'-gCC 3'	5'-TAC 3'	5'-gCg 3'	5'-AgT 3'	5'-CCg 3'	5'-ggC 3'	
						124	412					
						5'-gCA 3'	5'-ATA 3'					
						368						
						5'-gTC 3'						
						418						
						5'-Agg 3'						
3'-primer(s) ³	527	302	538	302	539	201	302	205	302	3 rd I	539	413
	5'-CCg 3'	5'-ggT 3'	5'-CCA 3'	5'-ggC 3'	5'-TCT 3'	5'-CTT 3'	5'-ggT 3'	5'-CCT 3'	5'-ggT 3'	5'-CTC 3'	5'-TCT 3'	5'-gCC 3'
	527	302				539	539	220	461			427
	5'-CCg 3'	5'-ggT 3'				5'-TCT 3'	5'-TCT 3'	5'-CgA 3'	5'-gCT 3'			5'-gTA 3'
								244	527			530
								5'-CTA 3'	5'-CCg 3'			5'-CCA 3'
								273				563
								5'-TTC 3'				5'-CgT 3'
								3 rd I				
								5'-gCA 3'				
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15	16	17	18	19	20	21	22	23
Length of spec. PCR product	130	210	85	165	180	120	100	95	445	85	95
	190		120	375	235	255	240	145	595	210	170
	240		145					210			
Length of int. pos. control ¹	1070	1070	1070	1070	1070	1070	800	1070	1070	800	1070
5'-primer(s) ²	126	368	97	361	201	126	361	368	379	524	201
	5'-ggA 3'	5'-gTg 3'	5'-TCg 3'	5'-AgA 3'	5'-CCA 3'	5'-ggA 3'	5'-AgT 3'	5'-gTT 3'	5'-ACg 3'	5'-CCA 3'	5'-CCA 3'
		368	124	736	666			436	527	3 rd I	
		5'-gTT 3'	5'-gCA 3'	5'-gCA 3'	5'-gAC 3'			5'-AgA 3'	5'-TgA 3'	5'-Cgg 3'	
			124					485	1019		
			5'-gCC 3'					5'-CAA 3'	5'-TgA 3'		
			156								
			5'-gTA 3'								
3'-primer(s) ³	214	539	201	3 rd I	341	205	413	539	3 rd I	3 rd I	257
	5'-CCA 3'	5'-TCT 3'	5'-CTT 3'	5'-CTC 3'	5'-CgT 3'	5'-CCT 3'	5'-gCC 3'	5'-TCT 3'	5'-gCA 3'	5'-CTC 3'	5'-CCT 3'
	273			861	861	341	430		1087	632	331
	5'-TTC 3'			5'-TCg 3'	5'-TCg 3'	5'-CgT 3'	5'-gCg 3'		5'-AgC 3'	5'-gTA 3'	5'-CTA 3'
	327						559				
	5'-TTT 3'						5'-CTC 3'				
							565				
							5'-CAT 3'				
Well No.	13	14	15	16	17	18	19	20	21	22	23

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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.

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CELL LINE VALIDATION SHEET																				
HLA-C*16 SSP primer set ²																				
				Well																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Prod. No.:	201675401	201908802	201554203	201554204	201554205	201554206	201554207	201898708	201554209	201554210	201554211	201554212	201898713	201554214	201675415	201675416
IHC cell line ¹		C*																		
1	9001 SA	*07:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
2	9280 LK707	*07:01	*15:05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
3	9011 E4181324	*12:02		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
4	9275 GU373	*03:04	*04:01	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
5	9009 KAS011	*06:02		-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM	*03:04	*07:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
7	9020 QBL	*05:01		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
8	9007 DEM	*04:01		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
9	9026 YAR	*12:03		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3	*01:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT	*16:01		+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*06:02		-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM	*01:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*01:02	*03:04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*03:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
17	9282 CTM3953540	*03:03	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
18	9257 32367	*01:02	*07:05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
19	9038 BM16	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
20	9059 SLE005	*03:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*12:03		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*01:02	*15:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*12:03		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*08:02		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
26	9285 WT49	*07:18		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
27	9191 CH1007	*07:04	*15:29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB	*05:01	*16:01	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+
29	9050 MOU	*16:01		+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*17:01		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
32	9297 HAG	*17:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*03:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*12:03		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*05:01		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
36	9024 KT17	*03:03	*04:01	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
37	9065 HHKB	*07:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
38	9099 LZL	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML	*02:02	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
40	9134 WHONP199	*01:02	*06:02	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*08:02		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
42	9066 TAB089	*01:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*01:02	*08:01	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
44	9057 TEM	*12:03		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*06:02	*17:01	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU	*07:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
47	9045 TUBO	*07:04	*15:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND	*04:01	*16:01	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+



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Lot-specific information

CELL LINE VALIDATION SHEET					Well							
HLA-C*16 SSP primer set ²					17	18	19	20	21	22	23	
				Prod. No.:	201675417	201554218	201554219	201554220	201908821	201554222	201554223	
	IHWC cell line ¹		C*									
1	9001 SA		*07:02		-	+	-	-	-	-	-	
2	9280 LK707		*07:01	*15:05	-	+	-	-	-	-	-	
3	9011 E4181324		*12:02		-	-	-	-	-	-	-	
4	9275 GU373		*03:04	*04:01	+	-	-	-	-	-	-	
5	9009 KAS011		*06:02		-	+	-	-	-	-	-	
6	9353 SM		*03:04	*07:02	-	+	-	-	-	-	-	
7	9020 QBL		*05:01		-	-	-	-	-	-	-	
8	9007 DEM		*04:01		+	-	-	-	-	-	-	
9	9026 YAR		*12:03		-	-	-	-	-	-	-	
10	9107 LKT3		*01:02		-	-	-	-	-	-	-	
11	9051 PITOUT		*16:01		-	-	-	-	-	-	-	
12	9052 DBB		*06:02		-	+	-	-	-	-	-	
13	9004 JESTHOM		*01:02		-	-	-	-	-	-	-	
14	9071 OLGA		*01:02	*03:04	-	-	-	-	-	-	-	
15	9075 DKB		*03:04		-	-	-	-	-	-	-	
16	9037 SWEIG007		*02:02		-	-	-	-	-	-	-	
17	9282 CTM3953540		*03:03	*07:01	-	+	-	-	-	-	-	
18	9257 32367		*01:02	*07:05	-	+	-	-	-	-	-	
19	9038 BM16		*07:01		-	+	-	-	-	-	-	
20	9059 SLE005		*03:04		-	-	-	-	-	-	-	
21	9064 AMALA		*03:03		-	-	-	-	-	-	-	
22	9056 KOSE		*12:03		-	-	-	-	-	-	-	
23	9124 IHL		*01:02	*15:02	-	-	-	-	-	-	-	
24	9035 JBUSH		*12:03		-	-	-	-	-	-	-	
25	9049 IBW9		*08:02		-	-	-	-	-	-	-	
26	9285 WT49		*07:18		-	+	-	-	-	-	-	
27	9191 CH1007		*07:04	*15:29	-	+	-	-	-	-	-	
28	9320 BEL5GB		*05:01	*16:01	-	-	-	-	-	-	-	
29	9050 MOU		*16:01		-	-	-	-	-	-	-	
30	9021 RSH		*17:01		-	-	-	-	-	-	-	
31	9019 DUCAF		*05:01		-	-	-	-	-	-	-	
32	9297 HAG		*17:03		-	-	-	-	-	-	-	
33	9098 MT14B		*03:04		-	-	-	-	-	-	-	
34	9104 DHIF		*12:03		-	-	-	-	-	-	-	
35	9302 SSTO		*05:01		-	-	-	-	-	-	-	
36	9024 KT17		*03:03	*04:01	+	-	-	-	-	-	-	
37	9065 HHKB		*07:02		-	+	-	-	-	-	-	
38	9099 LZL		*03:03		-	-	-	-	-	-	-	
39	9315 CML		*02:02	*07:01	-	+	-	-	-	-	-	
40	9134 WHONP199		*01:02	*06:02	-	+	-	-	-	-	-	
41	9055 H0301		*08:02		-	-	-	-	-	-	-	
42	9066 TAB089		*01:02		-	-	-	-	-	-	-	
43	9076 T7526		*01:02	*08:01	-	-	-	-	-	-	-	
44	9057 TEM		*12:03		-	-	-	-	-	-	-	
45	9239 SHJO		*06:02	*17:01	-	+	-	-	-	-	-	
46	9013 SCHU		*07:02		-	+	-	-	-	-	-	
47	9045 TUBO		*07:04	*15:02	-	+	-	-	-	-	-	
48	9303 TER-ND		*04:01	*16:01	+	-	-	-	-	-	-	

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Lot-specific information

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

²The 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 6 to 15 and 19 to 23 were available. The specificity of the primers in primer solutions 6 to 9, 11, 12, 14, 15 and 19 to 22 were tested by separately adding one or two additional 5'-primers, respectively one or two additional 3'-primers. In primer solution 10 it was only possible to test the 3'-primer, the 5'-primers were not possible to test. In primer solutions 13 and 23 it was only possible to test the 5'-primer, the 3'-primers were not possible to test. In primer solutions 1, 6, 7, 15 to 17, 20 and 21 one to four of the 5'-primers were not possible to test, and in primer solutions 2, 8, 9, 12, 18, 19 and 22 one to three of the 3'-primers were not possible to test. Additional primers in primer solutions 16 and 17 were tested by separately adding one additional 5'-primer.

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Lot-specific information

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Lot-specific information

ADDRESSES:

Manufacturer:

Caredx AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.

Tel: +46-8-508 939 00

Fax: +46-8-717 88 18

E-mail: orders-se@caredx.com

Web page: <https://labproducts.caredx.com/>

Distributed by:

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

Tel: +43-1-710 15 00

Fax: +43-1-710 15 00 10

E-mail: orders-at@caredx.com

Web page: <https://labproducts.caredx.com/>

CareDx Lab Solutions Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382

Tel: 1-877-653-78171

Fax: 610-344-7989

E-mail: orders-us@caredx.com

Web page: <https://labproducts.caredx.com/>

For information on CareDx distributors worldwide, contact **CareDx GmbH**.