101.432-12 – including *Taq* polymerase, IFU-01
101.432-12u – without *Taq* polymerase, IFU-02

Visit https://labproducts.caredx.com for **"Instructions for Use" (IFU)**

Lot No.: 7L9 **Lot-specific information**

Olerup SSP® HLA-A*33

Product number:	**101.432-12 – including *Taq* polymerase** **101.432-12u – without *Taq* polymerase**
Lot number:	**7L9**
Expiry date:	**2025-02-01**
Number of tests:	**12**
Number of wells per test:	**31+1**
Storage - pre-aliquoted primers:	**dark at -20ºC**
- PCR Master Mix:	**-20ºC**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 7L9.

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-A*33 LOT (8H5)

- The product documentation has been updated for new alleles of IMGT 3.42.0
- The kit resolution focuses on common and well documented (CWD) alleles[1].

[1]As described in section Uniquely Identified Alleles.

The HLA-A*33 primer set, specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup* SSP® HLA-A*33 lot was made **(Lot No. 8H5)**.

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

Well	5'-primer	3'-primer	rationale
12	Moved	Moved	Primer pairs moved to primer mixes 14 and 26 for improved HLA-specific amplification.
14	-	Added	3'-primer added from primer mix 12.
26	Added	Added	Primer pairs added from primer mix 12.
30	Exchanged	-	5'-primer exchanged for improved HLA-specific amplification.

[1]S. 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

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Lot No.: 7L9 **Lot-specific information**

Well **32** contains Negative Control primer pairs, that will amplify the 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 product generated by the positive control primer pair is 200 base pairs.

Length of PCR product	**105**	**200**	**105**	**80**	**75**	**80**	**85**
5'-primer[1]	**164**	**340**	**440**	**45**	**45**	**43**	**36**
	5'-CAC3'	**5'-Agg3'**	**5'-TTA3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-TAC3'**
							36
							5'-TAT3'
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**	**47**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**	**5'-ACA3'**
							48
							5'-gCA3'
							48
							5'-gCC3'
							52
							5'-TgT3'
A*	**+**	**+**	**+**				
B*	**+**	**+**	**+**				
C*	**+**	**+**	**+**				
DRB1				**+**	**+**		
DRB3				**+**	**+**		
DRB5				**+**			
DQB1					**+**		
DPB1						**+**	
DQA1							**+**

[1]The 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.
[2]The 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.

101.432-12 – including *Taq* polymerase, IFU-01
101.432-12u – without *Taq* polymerase, IFU-02

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Lot No.: 7L9 **Lot-specific information**

PRODUCT DESCRIPTION

HLA-A*33 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*33:01 to A*33:206 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 'HLA-A*33' in silver/gray ink.
Well No. 1 is marked with the Lot No. '7L9'.
Wells 1 to 31 – HLA-A*33 high resolution primers.
Well 32 – Negative Control (NC).
A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.
The PCR plates are covered with a PCR-compatible foil.
Please note: When removing each 32 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Due to the sharing of sequence motifs between HLA-A alleles non-HLA-A*33 alleles will be amplified by some primer mixes. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*33 alleles, i.e. **A*33:01 to A*33:206 alleles**, recognized by the HLA Nomenclature Committee in October 2020[1,2] will be amplified by the primers in the HLA-A*33 SSP kit[3].

The HLA-A*33 kit enables separation of the confirmed HLA-A*33 alleles as listed in the IMGT/HLA database 3.29.0. 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-A*33 alleles is listed below.

The HLA-A*33 kit also enables identification of many null and alternatively expressed alleles.

101.432-12 – including *Taq* polymerase, IFU-01 Visit https://labproducts.caredx.com for
101.432-12u – without *Taq* polymerase, IFU-02 **"Instructions for Use" (IFU)**

Lot No.: 7L9 **Lot-specific information**

The following HLA-A*33 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
A*33:03:03Q, 33:86	30	A*33:29, 33:39	20
A*33:15, 33:187	14	A*33:30, 33:73N	21
A*33:16, 33:64	15	A*33:31, 33:44	22
A*33:17, 33:140N	16	A*33:54, 33:164	24

[1]HLA-A alleles listed on the IMGT/HLA web page 2020-October-15, release 3.42.0, www.ebi.ac.uk/imgt/hla.

[2]Alleles 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.

[3]The following alleles give rise to identical amplification patterns with the HLA-A*33 subtyping kit. These alleles can be distinguished by e.g. the HLA-A low resolution kit and/or the respective subtyping kits.

Alleles	Alleles
A*33:08, A*31:99	A*33:51, 33:119, A*66:15
A*33:13, 33:183, A*26:207	A*33:184, A*26:177
A*33:18:01-33:18:02, A*29:105	

101.432-12 – including *Taq* polymerase, IFU-01
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Lot No.: 7L9 **Lot-specific information**

ALLELE CONFIRMATION STATUS

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

[1]Allele status "confirmed" or "unconfirmed" as listed on the IMGT/HLA web page 2017-August-10, release 3.29.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-A*33 homo- and heterozygotes is available upon request.

101.432-12 – including *Taq* polymerase, IFU-01
101.432-12u – without *Taq* polymerase, IFU-02

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Lot No.: 7L9 **Lot-specific information**

SPECIFICITY TABLE

HLA-A*33 SSP subtyping

Specificities and sizes of the PCR products of the 31+1 primer mixes used for HLA-A*33 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified HLA-A*33 alleles[3]	Other amplified HLA-A alleles
1	205 bp	**800 bp**	*33:01:01:01-33:01:15, 33:03:01:01-33:07, 33:10-33:20, 33:22-33:37, 33:39-33:50, 33:52, 33:54-33:68, 33:70-33:91, 33:93-33:108, 33:110-33:118, 33:120-33:206	*26:177, 26:207, 29:105, 68:29, 68:250
2	205 bp	**800 bp**	*33:01:01:01-33:01:15, 33:04-33:05, 33:07, 33:16, 33:27, 33:32:01-33:32:02, 33:34, 33:49-33:50, 33:64, 33:67-33:69, 33:89, 33:91-33:92, 33:109, 33:111, 33:121-33:122, 33:125, 33:127, 33:129N, 33:136, 33:143N, 33:157N, 33:165-33:168, 33:170-33:171, 33:180, 33:182, 33:191, 33:193, 33:196-33:197	*03:104, 31:88, 66:04
3[8]	155 bp	1070 bp	*33:01:01:01-33:01:15, 33:05, 33:07, 33:16, 33:27, 33:32:01-33:32:02, 33:34, 33:49-33:50, 33:64, 33:67-33:69, 33:89, 33:91-33:92, 33:109, 33:111, 33:121-33:122, 33:125, 33:127, 33:129N, 33:136, 33:143N, 33:157N, 33:165-33:168, 33:170-33:171, 33:180, 33:182, 33:191, 33:193, 33:196-33:197	*02:332, 03:104, 24:220, 31:88, 66:04
4	210 bp	1070 bp	*33:03:01:01-33:03:46, 33:06, 33:08-33:15, 33:17, 33:20-33:26, 33:28-33:31, 33:33, 33:35-33:37, 33:39-33:48, 33:51-33:63, 33:65-33:66, 33:70-33:88, 33:90,	*01:145, 02:41, 02:65, 02:80, 02:117, 02:135, 02:152, 02:289:01, 02:304, 02:309, 02:454, 02:829, 02:872, 03:103:01-03:103:02, 03:282, 11:116, 11:140, 11:372, 23:45, 24:62, 25:01:01:01-25:42N, 25:44-25:58, 25:60-25:70, 26:01:01:01-26:39, 26:41-26:43:02, 26:45-26:189,

			33:93-33:105, 33:107-33:108, 33:110, 33:112-33:120, 33:123N-33:124, 33:126, 33:128, 33:130-33:135, 33:137-33:142, 33:144-33:156N, 33:158-33:164, 33:169, 33:172-33:179, 33:181, 33:183-33:190, 33:192, 33:194N-33:195, 33:198N-33:206	26:191N-26:203, 26:205-26:207, 29:32, 31:01:02:01-31:02:02, 31:05, 31:07-31:68, 31:70-31:87, 31:89-31:178, 31:180-31:184N, 32:01:01:01-32:01:27, 32:01:29-32:03:01:02, 32:05-32:29, 32:31, 32:33:01-32:40, 32:42-32:47, 32:49-32:100, 32:102-32:118, 32:120-32:146, 34:01:01:01-34:01:05, 34:05-34:06, 34:11-34:12, 34:14, 34:16-34:19, 34:23, 43:01-43:02N, 66:01:01:01-66:03:01:03, 66:05-66:43, 74:01:01:01-74:39, **B*27:186**
5[4]	90 bp	**800 bp**	*33:04, 33:33	*26:68, **B*27:186**
6[4,5]	105 bp 170 bp	1070 bp	*33:20 *33:05, 33:58, 33:196	*31:94 *29:12, 29:92
7[4]	75 bp 105 bp 230 bp	1070 bp	*33:21, 33:53 *33:06 *33:51, 33:119	 *11:98, 11:250, 66:15, 68:04:01
8	160 bp 235 bp	1070 bp	*33:07 *33:24, 33:184	*02:444, 31:54 *02:243:01-02:243:03, 26:177, 29:19, 29:48
9[7]	135 bp 160 bp 185 bp	**800 bp**	*33:22 *33:14, 33:58 *33:01:02, 33:08-33:09	*02:24:02, 29:22, 31:99 *29:12, 29:92 *02:243:01-02:243:03, 29:02:23, 29:48
10[6,7]	135 bp 215 bp 285 bp	**800 bp**	*33:22 *33:08, 33:53, 33:183 *33:13, 33:184	*02:24:02, 29:22, 31:99 *02:243:01-02:243:03, 24:82, 29:48, 31:02:01-31:02:02, 31:07-31:08, 31:91, 31:109 *01:143, 11:43, 26:177, 26:207, 29:66, 31:03, 31:164, **C*07:449**
11[5]	165 bp	1070 bp	*33:10, 33:25	*01:01:114, 23:03:01, 24:21:03, 24:208:01, 29:03, 29:33, 31:05, 32:13
12	235 bp	1070 bp	*33:11, 33:125, 33:131, 33:147	*03:205, 11:43, 31:66, 31:89, 31:115, 68:29, 68:250
13[4,5]	95 bp 165 bp	1070 bp	*33:12 *33:25	
14[4]	120 bp 335 bp 390 bp	1070 bp	*33:15, 33:84 *33:19, 33:187 *33:80N	*02:140, 02:821, 26:99, 29:130, 31:48 *01:301Q, 02:10, 02:17:02:01-02:17:04, 02:39, 02:108, 02:110, 02:148, 02:242, 02:244, 02:268, 02:300, 02:303, 02:398, 02:453, 02:604, 02:617, 02:628, 02:630, 02:657, 02:680, 02:804, 03:15, 03:19, 03:347, 11:139, 24:04, 24:19, 24:28, 24:44, 24:89, 24:109, 24:129, 24:290, 24:406, 24:424, 29:07, 29:49, 31:29, *01:268, 02:789N, 11:137:01N, 26:60N, 74:32N
15[6]	140 bp 215 bp 255 bp	1070 bp	*33:16, 33:23 *33:65 *33:64	
16[4]	95 bp 140 bp 210 bp	1070 bp	*33:36 *33:140N *33:21	 *01:57N, 03:381N, 30:59N, 66:39N

			Lot-specific information	
	245 bp		*33:17	
17	145 bp	1070 bp	*33:18:01-33:18:02, 33:26	*01:244, 03:42, 03:133, 23:53, 23:70, 29:01:01:01-29:04, 29:06-29:31, 29:34, 29:36-29:50, 29:52-29:70, 29:72-29:76, 29:78N, 29:81, 29:84-29:86, 29:88-29:103, 29:105-29:131, 29:133:01:01-29:137, 29:139-29:146, 31:03-31:04:02, 31:06, 31:179, 32:30:01-32:30:02, 32:32, 68:168
	215 bp		*33:65	
18[4,6]	100 bp	**800 bp**	*33:27	
	145 bp		*33:57	
	240 bp		*33:82	
19[4]	120 bp	1070 bp	*33:28	*03:01:18, 03:22:02, 29:83, 29:132, 31:110, 32:10, 32:63, 32:145, 74:28, **B*15:02:07, B*15:17:03, C*02:02:15, C*03:03:20**
20[4]	95 bp	1070 bp	*33:29, 33:143N	
	225 bp		*33:39	*02:480, 02:924-02:925, 32:53, 68:176
21[4]	115 bp	1070 bp	*33:30	*02:342
	245 bp		*33:73N, 33:82, 33:129N	
22[4]	120 bp	1070 bp	*33:31, 33:84	*02:241, 02:684, 02:751, 26:24, 29:116
	255 bp		*33:44	
	545 bp		*33:123N	**C*01:171N**
23	180 bp	1070 bp	*33:32:01-33:32:02, 33:52	*02:332, 24:220
24[4]	95 bp	1070 bp	*33:34, 33:164	*01:01:114, 03:01:18, 11:01:28, 11:01:77, 24:21:03, 24:208:01, 29:09, 29:33, 29:144, 31:24, 32:33:01
	205 bp		*33:54	*29:59
25[5]	215 bp	1070 bp	*33:69, 33:83, 33:109	
	245 bp		*33:129N	
26[4]	100 bp	1070 bp	*33:36, 33:80N	*01:268, 11:137:01N, 26:60N, 74:32N
	150 bp		*33:77	
	190 bp		*33:74N, 33:111	*29:145, 31:132
27[5]	200 bp	1070 bp	*33:74N, 33:85, 33:92, 33:109	
28	230 bp	1070 bp	*33:70, 33:90	*01:84, 01:140:02, 02:214, 02:784, 03:145:02, 03:371, 11:54:01, **B*40:381, B*40:390, C*04:35:02**
	545 bp		*33:123N	**C*01:171N**
29[4]	105 bp	1070 bp	*33:42	*02:292
30	260 bp	1070 bp	*33:86	*03:265, 32:51, **B*18:173, B*44:287, C*07:756**
	545 bp		*33:03:03Q	*01:01:38L, 02:01:14Q, 11:01:98, 24:02:03Q, 30:02:25
31	155 bp	1070 bp	*33:96N	*01:52:01N, 26:107N
	220 bp		*33:90	*01:84, 02:214, 03:371
	260 bp		*33:68	*24:114, 29:61, 32:98
32[9]	-	-	**Negative Control**	

101.432-12 – including *Taq* polymerase, IFU-01 Visit https://labproducts.caredx.com for
101.432-12u – without *Taq* polymerase, IFU-02 **"Instructions for Use" (IFU)**

Lot No.: 7L9 **Lot-specific information**

[1]Alleles 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-A*33 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 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.

[2]The 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.

[3]For 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.

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

[5]Primer mixes 6, 11, 13, 25 and 27 may have tendencies of unspecific amplifications.

[6]Primer mixes 10, 15 and 18 have a tendency to giving rise to primer oligomer formation.

[7]Primer mixes 9 and 10 may give rise to a long unspecific amplification product of approximately 640 bp. This should be disregarded when interpreting the HLA-A*33 typings.

[8]Primer mix 3 may give rise to a lower yield of HLA-specific PCR product than the other A*33 primer mixes.

[9]Primer mix 32 contains a negative control, which will amplify the 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 product generated by the HGH positive control primer pair is 200 base pairs.

101.432-12 – including *Taq* polymerase, IFU-01
101.432-12u – without *Taq* polymerase, IFU-02

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Lot No.: 7L9 **Lot-specific information**

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	205	205	155	210	90	105	75	160	135	135	165	235
PCR product						170	105	235	160	215		
							230		185	285		
Length of int. pos. control[1]	800	800	1070	1070	800	1070	1070	1070	800	800	1070	1070
5'-primer(s)[2]	97	418	468	414	414	97	98	97	97	97	448	97
	5' -TCA 3'	5' -Agg 3'	5' -TCT 3'	5' -CAg 3'	5' -CAg 3'	5' -TCA 3'	5' -CTC 3'	5' -TCA 3'	5' -TCA 3'	5' -TCA 3'	5' -CCT 3'	5' -TCA 3'
				414		413	103	413	355	355	652	
				5' -CAg 3'		5' -CCA 3'	5' -CCT 3'	5' -CCA 3'	5' -CCg 3'	5' -CCg 3'	5' -CTg 3'	
							228					
							5' -ATg 3'					
							257					
							5' -CgA 3'					
3'-primer(s)[3]	259	583	583	583	463	221	290	292	218	270	570	290
	5' -gTT 3'	5' -gTg 3'	5' -gTg 3'	5' -gTA 3'	5' -gCT 3'	5' -ACA 3'	5' -CAA 3'	5' -gTg 3'	5' -gCC 3'	5' -ACT 3'	5' -CCg 3'	5' -CAg 3'
	260					233		530	221	341	778	
	5' -TgC 3'					5' -CCC 3'		5' -CCT 3'	5' -ACA 3'	5' -CgT 3'	5' -TgT 3'	
	265					475			240	448		
	5' -CCA 3'					5' -Cgg 3'			5' -ggA 3'	5' -CAA 3'		
									448			
									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.	95	120	140	95	145	100	120	95	115	120	180	95
PCR product	165	335	215	140	215	145		225	245	255		205
		390	255	210		240				545		
				245								
Length of int. pos. control[1]	1070	1070	1070	1070	1070	800	1070	1070	1070	1070	1070	1070
5'-primer(s)[2]	395	317	85	97	85	345	448	390	463	321	112	448
	5' -gCC 3'	5' -gCg 3'	5' -CCg 3'	5' -TCA 3'	5' -CCg 3'	5' -TT 3'	5' -CCT 3'	5' -g.C 3'	5' -TgT 3'	5' -g.C 3'	5' -CCT 3'	5' -CCT 3'
	652	652	158	355	161	390		397	629	652	453	
	5' -CTg 3'	5' -CTg 3'	5' -ggg 3'	5' -CCA 3'	5' -CgC 3'	5' -gAg 3'		5' -gCg 3'	5' -CAA 3'	5' -CTg 3'	5' -AAA 3'	
			370		413	632		649	629		453	
			5' -ATC 3'		5' -CCg 3'	5' -gAT 3'		5' -ACA 3'	5' -CAg 3'		5' -AAg 3'	
			482						632			
			5' -ggC 3'						5' -gAT 3'			
3'-primer(s)[3]	448	368	259	265	259	448	527	448	538	583	259	502
	5' -CAA 3'	5' -CAA 3'	5' -gTT 3'	5' -CCC 3'	5' -gTT 3'	5' -CAA 3'	5' -CCT 3'	5' -CAA 3'	5' -CAA 3'	5' -gTA 3'	5' -gTT 3'	5' -CTT 3'
	778	426	583	299	524	831		831	831	728	583	614
	5' -TgT 3'	5' -TCC 3'	5' -gTg 3'	5' -CCg 3'	5' -CAC 3'	5' -TCC 3'		5' -TCC 3'	5' -TCC 3'	5' -CCT 3'	5' -gTg 3'	5' -TgA 3'
		727		407						742		
		5' -CCA 3'		5' -ACT 3'						5' -CTC 3'		
		742		454						866		
		5' -CTC 3'		5' -CTA 3'						5' -gAT 3'		
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

101.432-12 – including *Taq* polymerase, IFU-01
101.432-12u – without *Taq* polymerase, IFU-02

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Lot No.: 7L9 **Lot-specific information**

Well No.	25	26	27	28	29	30	31
Length of spec.	215	100	200	230	105	260	155
PCR product	245	150		545		545	220
		190					260
Length of int.	1070	1070	1070	1070	1070	1070	1070
pos. control[1]							
5'-primer(s)[2]	94	355	95	321	277	28	365
	5' -ATC 3'	5' -CCA 3'	5' -TTC 3'	5' -g.C 3'	5' -AgT 3'	5' -TCC 3'	5' -gAC 3'
	95	652	97	391		3rd I	404
	5' -TTC 3'	5' -CTg 3'	5' -TCg 3'	5' -ACA 3'		5' -ATA 3'	5' -CCC 3'
	629		652	404			470
	5' -CAg 3'		5' -CTg 3'	5' -CCC 3'			5' -TTA 3'
	646						
	5' -ACA 3'						
3'-primer(s)[3]	259	407	259	583	341	118	583
	5' -gTT 3'	5' -ACT 3'	5' -gTT 3'	5' -gTA 3'	5' -Cgg 3'	5' -gCT 3'	5' -gTA 3'
	831	426	803			705	583
	5' -TCC 3'	5' -TCC 3'	5' -CCT 3'			5' -TCT 3'	5' -gTg 3'
		761	814				
		5' -Cgg 3'	5' -CAA 3'				
		803					
		5' -CCT 3'					
		806					
		5' -ACA 3'					
Well No.	25	26	27	28	29	30	31

[1]The 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.

[2]The 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.

[3]The 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.

101.432-12 – including *Taq* polymerase, IFU-01
101.432-12u – without *Taq* polymerase, IFU-02

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Lot No.: 7L9 **Lot-specific information**

CELL LINE VALIDATION SHEET

HLA-A*33 SSP subtyping kit[2]

					Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod No.:	202026801	202026802	202026803	202026804	202026805	202026806	202026807	202026808	202026809	202026810	202026811	202026812	202026813	202026814	202026815	202026816
	IHWC cell line[1]		A*	A*																
1	9001	SA	*24:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*30:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*02:01	*26:03	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*26:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
8	9025	DEU	*31:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*26:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*24:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*31:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*24:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*03:01	*80:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*33:03	*74:01	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*02:17		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
22	9056	KOSE	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*02:01	*34:01	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*32:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*33:01		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*02:05		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*24:10	*29:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*02:01	*29:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*30:01	*68:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*30:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*31:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*31:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*32:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*02:06	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*02:17		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
39	9315	CML	*01:01	*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*02:07	*30:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*02:07		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*02:06	*02:07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*66:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*23:01	*24:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*02:16	*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*02:01	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

101.432-12 – including *Taq* polymerase, IFU-01
101.432-12u – without *Taq* polymerase, IFU-02

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Lot No.: 7L9 **Lot-specific information**

CELL LINE VALIDATION SHEET

HLA-A*33 SSP subtyping kit[2]

					Well														
					17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
				Prod No.:	202026817	202026818	202026819	202026820	202026821	202026822	202026823	202026824	202026825	202026826	202026827	202026828	202026829	202026830	202026831
	IHWC cell line[1]		A*	A*															
1	9001	SA	*24:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*30:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*02:01	*26:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*26:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025	DEU	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*26:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*24:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*29:02		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*24:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*29:02		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*03:01	*80:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*33:03	*74:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*02:17		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*02:01	*34:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*32:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*33:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*02:05		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*24:10	*29:01	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*02:01	*29:02	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*29:02		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*30:01	*68:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*30:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*32:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*02:06	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*02:17		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*01:01	*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*02:07	*30:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*02:07		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*02:06	*02:07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*66:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*23:01	*24:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*02:16	*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*02:01	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

101.432-12 – including *Taq* polymerase, IFU-01 Visit https://labproducts.caredx.com for
101.432-12u – without *Taq* polymerase, IFU-02 **"Instructions for Use" (IFU)**

Lot No.: 7L9 **Lot-specific information**

[1]The 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.

[2]The 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 13, 15, 16, 18 to 21 and 23 to 29 and 31 were available. The specificities of the primers in primer solutions 5, 7 to 13, 19, 23, 24 and 27 were tested by separately adding additional 5'-primers and 3'-primers respectively. In primer solutions 16 and 26 it was only possible to test the 5'-primers, the 3'-primers were not possible to be tested. In primer solutions 15, 18, 20, 21, 25, 28, 29 and 31 it was only possible to test the 3'-primers, the 5'-primers were not possible to be tested. In primer solutions 7, 13, 17, 22, 23 and 27 one or two 5'-primers were not possible to be tested, and in primer solutions 1, 6, 8, 9, 11, 13, 14, 22, 24, 27 and 30 one or two 3'-primers were not possible to test. In addition, one or more primers in primer solutions 6, 14, 17, 22 and 30 were tested by separately adding one additional 5'-primer or one additional 3'-primer respectively.

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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**.