

101.421-06 – including *Taq* polymerase, IFU-01
101.421-06u – without *Taq* polymerase, IFU-02

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“Instructions for Use” (IFU)

Lot No.: **21Y**

Lot-specific Information
Olerup SSP® HLA-A*23

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Product number:	101.421-06 – including <i>Taq</i> polymerase 101.421-06u – without <i>Taq</i> polymerase
Lot number:	21Y
Expiry date:	2017-October-01
Number of tests:	6
Number of wells per test:	35+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. 21Y.

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*23 LOT (59V)

The HLA-A*23 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.

Four wells have been added to HLA-A*23, wells **33 to 36**.

¹As described in section Uniquely Identified Alleles.

The HLA-A*23 primer set, specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP®* HLA-A*23 lot was made (**Lot No. 59V**). The kit design is based on IMGT/HLA database 3.19.0.

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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.

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	Added	Added	Primer pair added for the A*23:69 allele.
14	-	Added	3'-primer added for the A*23:53 allele.
18	-	Added	3'-primer added for the A*23:01:16 allele.
19	Moved	Moved	Primer pair moved to well 35 for decreased tendency of primer oligomer formation.
27	-	Added	3'-primer added for the A*23:53 allele.
32	Added	Added	Updated negative control moved to well 36, primer pair added for the A*23:59 allele.
33	New	New	New primer pair added for the A*23:65 allele.
34	Added	Added	New primer pair added for the A*23:69 allele.
35	Added	Added	Primer pair added from well 19.
36	-	-	Updated negative control added from well 32.

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Well **36** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup SSP*® HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

HLA-specific PCR product sizes range from 75 to 200 base pairs.

The PCR product generated by the positive control primer pair is 430 base pairs.

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

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

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

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Lot-specific Information

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PRODUCT DESCRIPTION

HLA-A*23 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*23:01 to A*23:69 alleles.

PLATE LAYOUT

Each test consists of 36 PCR reactions in a 48 well cut PCR plate. Wells 37 to 48 are empty.

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	32
33	34	35	NC	empty	empty	empty	empty
empty	empty	empty	empty	empty	empty	empty	empty

The 48 well cut PCR plate is marked with ‘HLA-A*23’ in silver/gray ink.

Well No. 1 is marked with the Lot Number ‘21Y’.

Wells 1 to 35 – HLA-A*23 high resolution primers.

Well 36– 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 48 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*23 alleles will be amplified by primer mixes 1 to 10, 12 to 20, 22 to 30, 32 and 35. In addition, a few HLA-B and HLA-C alleles will be amplified by primer mixes 1, 3, 10, 14, 15 and 18.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*23 alleles, i.e. **A*23:01 to A*23:69 alleles**, recognized by the HLA Nomenclature Committee in January 2015^{1,2} will be amplified by the primers in the HLA-A*23 subtyping kit.

The HLA-A*23 kit enables separation of the confirmed HLA-A*23 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-A*23 alleles is listed below.

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The HLA-A*23 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles

The HLA-A*23 subtyping kit cannot distinguish the silent mutations in the A*23:01:01-23:01:15 alleles, the A*23:03:01-23:03:02 alleles and the A*23:37:01-23:37:02 alleles.

The following HLA-A*23 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
A*23:08N, 23:22	7	A*23:16, 23:29	20
A*23:09, 23:26	8	A*23:18, 23:28	4
A*23:10, 23:23	10	A*23:41, 23:42	25
A*23:13, 23:33	17		

¹HLA-A alleles listed on the IMGT/HLA web page 2015-January-19, release 3.19.0, 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>.

ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
A*23:01:01	Confirmed	A*23:14:02	Unconfirmed	A*23:43	Confirmed
A*23:01:02	Confirmed	A*23:15	Confirmed	A*23:44	Unconfirmed
A*23:01:03	Confirmed	A*23:16	Unconfirmed	A*23:45	Unconfirmed
A*23:01:04	Confirmed	A*23:17	Confirmed	A*23:46	Unconfirmed
A*23:01:05	Unconfirmed	A*23:18	Unconfirmed	A*23:47	Confirmed
A*23:01:06	Confirmed	A*23:19Q	Confirmed	A*23:48	Confirmed
A*23:01:07	Unconfirmed	A*23:20	Unconfirmed	A*23:49	Confirmed
A*23:01:08	Unconfirmed	A*23:21	Confirmed	A*23:50	Unconfirmed
A*23:01:09	Confirmed	A*23:22	Unconfirmed	A*23:51	Unconfirmed
A*23:01:10	Confirmed	A*23:23	Unconfirmed	A*23:52	Unconfirmed
A*23:01:11	Unconfirmed	A*23:24	Confirmed	A*23:53	Confirmed
A*23:01:12	Unconfirmed	A*23:25	Unconfirmed	A*23:54	Unconfirmed
A*23:01:13	Unconfirmed	A*23:26	Unconfirmed	A*23:55	Unconfirmed
A*23:01:14	Unconfirmed	A*23:27	Confirmed	A*23:56	Confirmed
A*23:01:15	Unconfirmed	A*23:28	Confirmed	A*23:57	Unconfirmed
A*23:01:16	Unconfirmed	A*23:29	Unconfirmed	A*23:58	Unconfirmed
A*23:02	Unconfirmed	A*23:30	Confirmed	A*23:59	Confirmed
A*23:03:01	Unconfirmed	A*23:31	Confirmed	A*23:60	Unconfirmed
A*23:03:02	Unconfirmed	A*23:32	Unconfirmed	A*23:61	Unconfirmed
A*23:04	Confirmed	A*23:33	Unconfirmed	A*23:62	Unconfirmed
A*23:05	Confirmed	A*23:34	Unconfirmed	A*23:63	Unconfirmed
A*23:06	Confirmed	A*23:35	Unconfirmed	A*23:64	Unconfirmed
A*23:07N	Unconfirmed	A*23:36	Unconfirmed	A*23:65	Confirmed
A*23:08N	Confirmed	A*23:37:01	Unconfirmed	A*23:66	Unconfirmed
A*23:09	Unconfirmed	A*23:37:02	Unconfirmed	A*23:67	Unconfirmed
A*23:10	Unconfirmed	A*23:38N	Unconfirmed	A*23:68	Unconfirmed
A*23:11N	Unconfirmed	A*23:39	Unconfirmed	A*23:69	Unconfirmed
A*23:12	Confirmed	A*23:40	Unconfirmed		
A*23:13	Unconfirmed	A*23:41	Unconfirmed		
A*23:14:01	Unconfirmed	A*23:42	Unconfirmed		

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

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-A*23 homo- and heterozygotes is available upon request.

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Lot-specific Information
SPECIFICITY TABLE

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HLA-A*23 SSP subtyping

Specificities and sizes of the PCR products of the 35+1 primer mixes used for HLA-A*23 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*23 alleles ³	Other amplified HLA Class I alleles ⁴
1	210 bp	800 bp	*23:01:01-23:01:16, 23:03:01-23:65, 23:67- 23:69	*02:17:01-02:17:03, 02:108, 02:110, 02:268, 02:300, 02:303, 24:13:01-24:13:02, 24:18, 24:24, 24:94, 24:188, 24:207, 24:228, 29:07, 29:49, 31:29, B*18:27
2	160 bp	1070 bp	*23:01:01-23:02, 23:05- 23:42, 23:44-23:69	*02:19, 02:36-02:37, 02:54, 02:255, 02:417, 11:209, 24:02:01:01-24:02:41, 24:02:43- 24:02:69, 24:02:71-24:02:91, 24:04-24:09N, 24:11N, 24:13:01-24:15, 24:17, 24:19- 24:20, 24:24-24:32, 24:34-24:64, 24:66- 24:74:02, 24:76-24:93, 24:95-24:109, 24:111-24:124, 24:126-24:137, 24:139- 24:157, 24:159-24:166, 24:168-24:203, 24:205-24:206, 24:209, 24:212-24:218, 24:220-24:282, 24:284-24:288, 24:290- 24:294Q, 33:19, 68:26, 68:65, 68:115
3 ⁵	125 bp	800 bp	*23:03:01-23:03:02	*11:139, 24:21:01, 24:208, 29:07, 29:49, 31:29, B*18:27
4 ⁵	270 bp	1070 bp	*23:25	*24:267
	90 bp		*23:18	
	170 bp		*23:58	
5	200 bp	800 bp	*23:28	*24:77
	230 bp		*23:06	*31:36
6	470 bp	1070 bp	*23:07N	*01:04N, 03:21N, 11:21N, 24:11N
7 ⁵	95 bp	800 bp	*23:08N	*02:82N
	145 bp		*23:39	*30:22
	205 bp		*23:22	
8 ⁷	140 bp	1070 bp	*23:26, 23:39	*30:22
	170 bp		*23:48	
	215 bp		*23:09	*01:02, 01:20, 24:129
9	235 bp	1070 bp	*23:05	*24:25
10	135 bp	800 bp	*23:23	B*18:27
	240 bp		*23:10, 23:43	*11:139 ^w , 24:10:01 ^w -24:10:02 ^w , 24:46, 24:210 ^w
11	200 bp	800 bp	*23:11N	
	270 bp		*23:32	
12	135 bp	1070 bp	*23:69	
	190 bp		*23:12	*24:30, 24:42, 25:11, 32:08
13 ⁵	90 bp	800 bp	*23:30	
	210 bp		*23:02, 23:24, 23:66	*24:06, 24:87, 24:138, 24:167, 24:285, 24:289

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14⁵	85 bp 245 bp	1070 bp	*23:53 *23:04	*11:139, 24:17, 24:41, 24:208, 29:07, 29:49 *02:17:01-02:17:03, 02:108, 02:110, 02:268, 02:300, 02:303, 11:139, 24:03:01- 24:03:02, 24:10:01-24:10:02, 24:18, 24:22, 24:33, 24:94, 24:125, 24:138, 24:167, 24:204, 24:207, 24:210, 29:07, 29:49, 31:29, B*18:27
15	210 bp 290 bp	1070 bp	*23:01:16, 23:14:01 *23:47	*24:13:02, B*18:27 *24:234
16	175 bp 205 bp	800 bp	*23:14:01-23:14:02	*02:17:01 ^W -02:17:03 ^W , 11:139, 24:02:01:01- 24:04, 24:06-24:11N, 24:13:01-24:13:02, 24:17-24:23, 24:25-24:50, 24:54-24:56, 24:58-24:63, 24:66-24:91, 24:93, 24:95- 24:113, 24:115-24:129, 24:131-24:137, 24:139-24:167, 24:169-24:187, 24:189- 24:193, 24:195-24:198, 24:200-24:210, 24:212-24:221, 24:223-24:227, 24:229- 24:290, 24:292, 24:294Q, 33:19 *11:139, 24:02:01:01-24:02:15, 24:02:17- 24:05, 24:07:01-24:11N, 24:17, 24:19- 24:21:02, 24:23-24:50, 24:55-24:56, 24:58- 24:63, 24:66-24:86N, 24:88-24:90N, 24:93, 24:95-24:106, 24:108-24:113, 24:115- 24:132N, 24:134-24:137, 24:139-24:166, 24:168-24:187, 24:189-24:206, 24:208- 24:210, 24:212-24:221, 24:223-24:226:02, 24:229-24:284, 24:286-24:288, 24:290, 24:292-24:294Q, 26:16, 33:19, 68:45, 68:117
17⁵	125 bp 225 bp	1070 bp	*23:33 *23:13	*03:72, 11:88, 24:07:01-24:07:02, 24:19, 24:24, 24:131, 24:288, 24:290, 24:294Q, 29:37, 29:56, 30:01:01-30:01:10, 30:11:01- 30:11:02, 30:14L-30:20, 30:23-30:26, 30:30-30:31, 30:35-30:43, 30:48-30:49, 30:52-30:54, 30:56, 30:58-30:60, 30:62- 30:63, 30:65, 30:72-30:75, 30:78N, 30:81- 30:83, 30:86-30:89, 30:91-30:93, 68:45, 68:117
18⁵	120 bp	1070 bp	*23:01:01-23:01:16, 23:02 ^W , 23:04-23:23, 23:25-23:33, 23:35-23:56, 23:58-23:65, 23:67-23:69	*02:01:22, 02:06:04, 02:40:01-02:40:02, 02:51, 02:130, 02:171:01, 24:13:02, 24:24, 31:67-31:68, 32:28, 32:66, 33:32:01, 68:08:01, 68:51 ^W , B*51:136, C*07:204
19⁵	120 bp 195 bp	800 bp	*23:15 *23:46	*24:66, 30:75
20	130 bp 195 bp 230 bp 270 bp	800 bp	*23:29 *23:46 *23:16 *23:32	*24:128 *24:66, 30:75

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21⁵	90 bp	800 bp	*23:01:01-23:01:16, 23:02 [?] -23:04 [?] , 23:06- 23:07N, 23:08N [?] , 23:09, 23:10 [?] -23:16 [?] , 23:18 [?] , 23:19Q, 23:20 [?] -23:25 [?] , 23:26, 23:27 [?] -23:37:02 [?] , 23:38N, 23:39 [?] -23:68 [?] , 23:69	
22^{5,6}	90 bp	1070 bp	*23:31, 23:45, 23:53	*11:139, 24:17, 24:41, 24:62, 24:106, 24:208, 29:07, 29:49, 31:29
23^{5,7}	290 bp 80 bp	1070 bp	*23:19Q *23:45	*02:41, 02:80, 02:117, 02:289:01, 02:304, 02:454, 24:62, 26:10, 31:67-31:68, 32:28, 32:66, 33:32:01
24⁵	170 bp 105 bp 180 bp 240 bp	800 bp	*23:20, 23:58 *23:49 *23:21 *23:40	*30:06
25⁵	95 bp 205 bp	800 bp	*23:41 *23:42	*02:221, 29:15, 31:78
26⁵	80 bp	1070 bp	*23:36	*24:32, 32:05
27^{5,6}	85 bp 200 bp	1070 bp	*23:53 *23:34, 23:57	*11:139, 24:17, 24:41, 24:208, 29:07, 29:49 *02:17:01-02:17:03, 02:108, 02:110, 02:268, 02:300, 02:303, 24:13:01, 24:94, 24:188, 24:207, 24:228
28	285 bp 385 bp	800 bp	*23:35 *23:02 [?] -23:04 [?] , 23:05, 23:08N [?] , 23:10 [?] -23:16 [?] , 23:17, 23:18 [?] , 23:20 [?] - 23:25 [?] , 23:27 [?] -23:37:02 [?] , 23:39 [?] -23:68 [?]	*24:02:01:01-24:02:09, 24:02:11-24:04, 24:05 [?] -24:06 [?] , 24:07:01-24:10:01, 24:10:02 [?] , 24:11N, 24:13:01 [?] -24:15 [?] , 24:17 [?] -24:19 [?] , 24:20, 24:21:01 [?] -24:21:02 [?] , 24:22, 24:23 [?] -24:28 [?] , 24:29, 24:30 [?] -24:32 [?] , 24:34 [?] -24:43 [?] , 24:44, 24:45N [?] -24:60N [?] , 24:61, 24:62 [?] -24:64 [?] , 24:66 [?] -24:78 [?] , 24:79, 24:80 [?] -24:85 [?] , 24:86N, 24:87 [?] -24:94 [?] , 24:95, 24:96 [?] -24:127 [?] , 24:128-24:129, 24:130 [?] -24:131 [?] , 24:132N, 24:133 [?] - 24:141 [?] , 24:142-24:143, 24:144 [?] -24:151 [?] , 24:152, 24:153 [?] -24:162 [?] , 24:163N, 24:164 [?] - 24:193 [?] , 24:194, 24:195 [?] -24:210 [?] , 24:212 [?] - 24:214 [?] , 24:215, 24:216 [?] -24:225:01 [?] , 24:225:02, 24:226:01 [?] , 24:226:02, 24:227 [?] - 24:230 [?] , 24:231, 24:232N [?] , 24:233-24:235, 24:236 [?] -24:247 [?] , 24:248-24:251, 24:252N [?] - 24:253 [?] , 24:254-24:256, 24:257 [?] -24:258 [?] , 24:259-24:273, 24:274 [?] -24:277 [?] , 24:278N, 24:279 [?] -24:285 [?] , 24:286, 24:287 [?] -24:293 [?] , 24:294Q, 33:19 [?]
29	170 bp 200 bp 240 bp 290 bp	1070 bp	*23:48 *23:37:01-23:37:02 *23:40 *23:47	*24:273 *24:234
30⁵	110 bp 245 bp	1070 bp	*23:56 *23:38N	*24:61
31	185 bp	1070 bp	*23:44	

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32 ⁵	85 bp	1070 bp	*23:59	*11:166, 30:56, 31:85, 80:01:01:01-80:03
33	165 bp	1070 bp	*23:65	
34	135 bp	1070 bp	*23:69	
35 ⁵	105 bp	800 bp	*23:49	*30:06
	260 bp		*23:27	*24:255
36 ⁸	-	-	Negative Control	

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*23 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.

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 Due to the sharing of sequence motifs between HLA-A alleles non-HLA-A*23 alleles will be amplified by primer mixes 1 to 10, 12 to 20, 22 to 30, 32 and 35. In addition, a few HLA-B and HLA-C alleles will be amplified by primer mixes 1, 3, 10, 14, 15 and 18.

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

⁶ Primer mixes 22 and 27 may give rise to a lower yield of HLA-specific PCR product than the other A*23 primer mixes.

⁷ Primer mix 8 and 23 may have tendencies of unspecific amplifications.

⁸ Primer mix 36 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by the control primer pairs matching the human growth hormone gene. HLA-specific PCR product sizes range from 75 to 200 base pairs and the PCR product generated by the HGH positive control primer pair is 430 base pairs.

‘w’, may be weakly amplified.

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

101.421-06 – including *Taq* polymerase, IFU-01
101.421-06u – without *Taq* polymerase, IFU-02

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“Instructions for Use” (IFU)

Lot No.: 21Y

Lot-specific Information

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	210	160	125	90	230	470	95	140	235	135	200	135
PCR product			270	170			145	170		240	270	190
				200			205	215				
Length of int. pos. control ¹	800	1070	800	1070	800	1070	800	1070	1070	800	800	1070
5'-primer(s) ²	368	453	368	98	144	3 rd I	98	98	28	368	90	144
	5'-gTT 3'	5'-AAA 3'	5'-gTT 3'	5'-CTC 3'	5'-gCC 3'	5'-ATA 3'	5'-CTC 3'	5'-CTC 3'	5'-TCg 3'	5'-gTT 3'	5'-AgT 3'	5'-gCC 3'
				678			564				160	920
				5'-AgA 3'			5'-TgA 3'				5'-ACg 3'	5'-CCA 3'
3'-primer(s) ³	539	570	453	256	331	621	200	193	92	463	317	292
	5'-TCA 3'	5'-CCg 3'	5'-TCg 3'	5'-CTg 3'	5'-CTC 3'	5'-ggg 3'	5'-TCC 3'	5'-CgA 3'	5'-AAC 3'	5'-gCT 3'	5'-ggA 3'	5'-gTg 3'
			595	728			262	200		559		1013
			5'-CCg 3'	5'-CCT 3'			5'-TgC 3'	5'-TCC 3'		5'-CCg 3'		5'-CTT 3'
				809			616	227		571		
				5'-CAA 3'			5'-CgT 3'	5'-CTg 3'		5'-CCT 3'		
								271				
								5'-CAT 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.	90	85	210	175	125	120	120	130	90	90	80	105
PCR product	210	245	290	205	225		195	195		290	170	180
								230				240
								270				
Length of int. pos. control ¹	800	1070	1070	800	1070	1070	800	800	800	1070	1070	800
5'-primer(s) ²	368	368	368	98	98	453	418	90	920	368	414	98
	5'-gTT 3'	5'-gTT 3'	5'-gTT 3'	5'-CTC 3'	5'-CTC 3'	5'-AAA 3'	5'-AgC 3'	5'-AgT 3'	5'-CCA 3'	5'-gTT 3'	5'-CAg 3'	5'-CTC 3'
				368			493	228			678	
				5'-gTT 3'			5'-CTg 3'	5'-ATg 3'			5'-AgA 3'	
								379				
								5'-ACA 3'				
								418				
								5'-AgC 3'				
3'-primer(s) ³	419	413	538	259	181	524	570	317	968	418	453	163
	5'-CgC 3'	5'-gCC 3'	5'-CAg 3'	5'-gTT 3'	5'-gTA 3'	5'-CAC 3'	5'-CCg 3'	5'-ggA 3'	5'-CAg 3'	5'-gTC 3'	5'-TCT 3'	5'-CgC 3'
	530	570	616	502	282	538		570		619	806	238
	5'-CCA 3'	5'-CAC 3'	5'-CgC 3'	5'-CTT 3'	5'-gAC 3'	5'-CAg 3'		5'-CCg 3'		5'-gTT 3'	5'-CTA 3'	5'-CCT 3'
	539			539	282						809	299
	5'-TCC 3'			5'-TCT 3'	5'-gAC 3'						5'-CAA 3'	5'-TCg 3'
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

101.421-06 – including *Taq* polymerase, IFU-01
101.421-06u – without *Taq* polymerase, IFU-02

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Lot No.: 21Y

Lot-specific Information

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Well No.	25	26	27	28	29	30	31	32	33	34	35
Length of spec. PCR product	95	80	85	385	170	110	185	85	165	135	105
			200		200	245					260
			285		240						
					290						
Length of int. pos. control ¹	800	1070	1070	800	1070	1070	1070	1070	1070	1070	800
5'-primer(s) ²	376	265	368	678	98	98	395	176	453	920	98
	5'-gCT 3'	5'-CAg 3'	5'-gTT 3'	5'-AgA 3'	5'-CTC 3'	5'-CTC 3'	5'-gCA 3'	5'-gCA 3'	5'-AAA 3'	5'-CCA 3'	5'-CTC 3'
	484				368						
	5'-ACg 3'				5'-gTT 3'						
3'-primer(s) ³	538	302	413	920	227	167	538	218	577	1013	163
	5'-CAA 3'	5'-ggC 3'	5'-gCC 3'	5'-Tgg 3'	5'-CTg 3'	5'-ACC 3'	5'-CAA 3'	5'-gCg 3'	5'-gCC 3'	5'-CTT 3'	5'-CgC 3'
			524		255	303					316
			5'-CAT 3'		5'-TCC 3'	5'-AgT 3'					5'-gAA 3'
			534		255						
			5'-CgT 3'		5'-TCT 3'						
			614		299						
			5'-Tgg 3'		5'-TCg 3'						
					616						
					5'-CgC 3'						
Well No.	25	26	27	28	29	30	31	32	33	34	35

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.

101.421-06 – including *Taq* polymerase, IFU-01
101.421-06u – without *Taq* polymerase, IFU-02

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Lot No.: **21Y**

Lot-specific Information

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CELL LINE VALIDATION SHEET																						
HLA-A*23 SSP subtyping kit²																						
					Lot No.:	Well																
						1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
						201550401	201550402	201550403	201550404	201550405	201550406	201550407	201550408	201550409	201550410	201550411	201550412	201550413	201550414	201550415	201550416	
	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				+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	W
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			-	-	-	-	-	-	-	-	-	W	-	-	-	+	-	-	+
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				+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	W
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			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

101.421-06 – including Taq polymerase, IFU-01
101.421-06u – without Taq polymerase, IFU-02

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Lot No.: 21Y

Lot-specific Information

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CELL LINE VALIDATION SHEET																			
HLA-A*23 SSP subtyping kit ²																			
				Well															
				17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
				201550417	201550418	201550419	201550420	201550421	201550422	201550423	201550424	201550425	201550426	201550427	201550428	201550429	201550430	201550431	201550432
	IHWC cell line ¹	A*	A*	Lot No.:															
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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

101.421-06 – including *Taq* polymerase, IFU-01
101.421-06u – without *Taq* polymerase, IFU-02

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Lot No.: **21Y**

Lot-specific Information

www.olerup-ssp.com

CELL LINE VALIDATION SHEET								
HLA-A*23 SSP subtyping kit²								
					Well			
					33	34	35	
					Lot No.:	201550433	201550434	201550435
	IHWC cell line¹	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		-	-	-	

101.421-06 – including Taq polymerase, IFU-01
101.421-06u – without Taq polymerase, IFU-02

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Lot No.: 21Y

Lot-specific Information

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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 3 to 5, 9, 11, 13, 15, 19, 20, 23 to 26, 29 to 31 and 33 to 35 were available.

The specificities of the primers in primer solutions 3, 13, 15, 19, 20, 23 and 26 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer.

In primer solutions 4, 5, 9, 24, 29, 30 and 33 to 35 it was only possible to test the 5'-primers, the 3'-primers were not possible to test.

In primer solutions 11, 25 and 31 it was only possible to test the 3'-primers, the 5'-primers was not possible to test.

In primer solutions 3, 7, 8, 10, 12, 13, 15, 17, 22, 23 and 27 one or two 3'-primers were not possible to test, and in primer solutions 19, 20 and 23 one or three 5'-primer were not possible to test. Additional primers in primer solutions 7, 8, 10, 12, 14, 16 and 27 were tested by separately adding one additional 5'-primer and/or one additional 3'-primer.

101.421-06 – including *Taq* polymerase, IFU-01
101.421-06u – without *Taq* polymerase, IFU-02

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Lot-specific Information

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