

101.430-12 – including *Taq* polymerase, IFU-01
101.430-12u – without *Taq* polymerase, IFU-02

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“Instructions for Use” (IFU)

Lot No.: **6G7**

Lot-specific Information
Olerup SSP[®] HLA-A*31

Product number:	101.430-12 – including <i>Taq</i> polymerase 101.430-12u – without <i>Taq</i> polymerase
Lot number:	6G7
Expiry date:	2021-02-01
Number of tests:	12
Number of wells per test:	41+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. 6G7.

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*31 LOT (4F2)**

The HLA-A*31 kit is updated for new alleles to enable separation of:

- Null and Alternatively expressed alleles
- The product documentation has been updated for new alleles of IMGT 3.32

Two wells have been added to HLA-A*31, wells **41 and 42**.

The format of the Worksheet has been changed.

The HLA-A*31 primer set, specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP[®]* HLA-A*31 lot was made (**Lot No. 4F2**).

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

Well	5'-primer	3'-primer	rationale
40	Added	Added	Negative Control moved to well 42, new primer pair added for the A*31:126N allele.
41	New	New	New primer pair added for the A*31:131N allele.
42	-	-	Negative Control Added from well 40.

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Well **42** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup SSP*[®] HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

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

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

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

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

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

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Lot-specific Information

PRODUCT DESCRIPTION

HLA-A*31 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*31:01 to A*31:135 alleles.

PLATE LAYOUT

Each test consists of 42 PCR reactions in a 48 well cut PCR plate. Wells 43 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	36	37	38	39	40
41	NC	empty	empty	empty	empty	empty	empty

The 48 well cut PCR plate is marked with ‘HLA-A*31’ in silver/gray ink.

Well No. 1 is marked with the Lot Number ‘6G7’.

Wells 1 to 41 – HLA-A*31 high resolution primers.

Well 42 – 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*31 alleles will be amplified by some primer mixes. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*31 alleles, i.e. **A*31:01 to A*31:135 alleles**, recognized by the HLA Nomenclature Committee in April 2018^{1,2} will be amplified by the primers in the HLA-A*31 subtyping kit³.

The HLA-A*31 kit enables separation of the confirmed HLA- A*31 alleles as listed in the IMGT/HLA database 3.28.0. 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*31 alleles is listed below.

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The HLA-A*31 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.

The following HLA-A*31 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
A*31:01:02:03N, 31:35	21	A*31:29, 31:59	25
A*31:16, 31:46	16	A*31:36, 31:48	29

¹HLA-A alleles listed on the IMGT/HLA web page 2018-April-16, release 3.32.0, 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 A*31:44 and the A*33:15 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 29.

The A*31 primer set cannot separate the A*31:89 from the A*33:125 and A*33:131 alleles. These alleles can be distinguished by the HLA-A low resolution and/or HLA-A*33 kits.

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Lot-specific Information

ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
A*31:01:02:01	Confirmed	A*31:13	Unconfirmed	A*31:53	Unconfirmed	A*31:93	Unconfirmed
A*31:01:02:02	Unconfirmed	A*31:14N	Unconfirmed	A*31:54	Confirmed	A*31:94	Unconfirmed
A*31:01:02:03N	Unconfirmed	A*31:15	Confirmed	A*31:55	Unconfirmed	A*31:95	Unconfirmed
A*31:01:02:04	Confirmed	A*31:16	Confirmed	A*31:56	Confirmed	A*31:96	Confirmed
A*31:01:02:05	Unconfirmed	A*31:17	Confirmed	A*31:57	Unconfirmed	A*31:97	Unconfirmed
A*31:01:02:06	Unconfirmed	A*31:18	Confirmed	A*31:58	Unconfirmed	A*31:98	Confirmed
A*31:01:03	Unconfirmed	A*31:19	Confirmed	A*31:59	Unconfirmed	A*31:99	Unconfirmed
A*31:01:04	Confirmed	A*31:20	Confirmed	A*31:60N	Unconfirmed	A*31:100	Unconfirmed
A*31:01:05	Unconfirmed	A*31:21	Unconfirmed	A*31:61	Unconfirmed	A*31:101	Unconfirmed
A*31:01:06	Unconfirmed	A*31:22	Confirmed	A*31:62	Unconfirmed	A*31:102	Confirmed
A*31:01:07	Unconfirmed	A*31:23	Unconfirmed	A*31:63	Unconfirmed	A*31:103	Unconfirmed
A*31:01:08	Unconfirmed	A*31:24	Confirmed	A*31:64	Unconfirmed	A*31:104	Unconfirmed
A*31:01:09	Unconfirmed	A*31:25	Unconfirmed	A*31:65	Unconfirmed	A*31:105	Confirmed
A*31:01:10	Unconfirmed	A*31:26	Confirmed	A*31:66	Unconfirmed	A*31:106	Unconfirmed
A*31:01:11	Unconfirmed	A*31:27	Confirmed	A*31:67	Unconfirmed	A*31:107	Unconfirmed
A*31:01:12	Unconfirmed	A*31:28	Confirmed	A*31:68	Confirmed	A*31:108	Unconfirmed
A*31:01:13	Unconfirmed	A*31:29	Unconfirmed	A*31:69	Unconfirmed	A*31:109	Unconfirmed
A*31:01:14	Unconfirmed	A*31:30	Unconfirmed	A*31:70	Unconfirmed	A*31:110	Unconfirmed
A*31:01:15	Unconfirmed	A*31:31	Confirmed	A*31:71	Unconfirmed	A*31:111	Confirmed
A*31:01:16	Unconfirmed	A*31:32	Unconfirmed	A*31:72	Unconfirmed	A*31:112	Unconfirmed
A*31:01:17	Unconfirmed	A*31:33	Unconfirmed	A*31:73	Unconfirmed	A*31:113	Unconfirmed
A*31:01:18	Unconfirmed	A*31:34	Unconfirmed	A*31:74	Unconfirmed	A*31:114	Unconfirmed
A*31:01:19	Unconfirmed	A*31:35	Unconfirmed	A*31:75	Unconfirmed	A*31:115	Unconfirmed
A*31:01:20	Unconfirmed	A*31:36	Confirmed	A*31:76	Unconfirmed	A*31:116	Unconfirmed
A*31:01:21	Unconfirmed	A*31:37	Unconfirmed	A*31:77	Unconfirmed	A*31:117	Unconfirmed
A*31:01:22	Unconfirmed	A*31:38	Confirmed	A*31:78	Unconfirmed	A*31:118	Unconfirmed
A*31:01:23	Unconfirmed	A*31:39	Confirmed	A*31:79	Unconfirmed	A*31:119	Unconfirmed
A*31:01:24	Confirmed	A*31:40	Confirmed	A*31:80	Unconfirmed	A*31:120	Unconfirmed
A*31:01:25	Unconfirmed	A*31:41	Confirmed	A*31:81	Unconfirmed	A*31:121	Unconfirmed
A*31:02	Confirmed	A*31:42	Unconfirmed	A*31:82	Unconfirmed	A*31:122	Unconfirmed
A*31:03	Unconfirmed	A*31:43	Confirmed	A*31:83	Unconfirmed	A*31:123	Unconfirmed
A*31:04	Confirmed	A*31:44	Confirmed	A*31:84	Unconfirmed	A*31:124	Unconfirmed
A*31:05	Confirmed	A*31:45	Unconfirmed	A*31:85	Unconfirmed	A*31:125	Unconfirmed
A*31:06	Confirmed	A*31:46	Unconfirmed	A*31:86	Unconfirmed		
A*31:07	Unconfirmed	A*31:47	Unconfirmed	A*31:87	Unconfirmed		
A*31:08	Confirmed	A*31:48	Confirmed	A*31:88	Unconfirmed		
A*31:09	Confirmed	A*31:49	Unconfirmed	A*31:89	Unconfirmed		
A*31:10	Unconfirmed	A*31:50	Unconfirmed	A*31:90	Unconfirmed		
A*31:11	Confirmed	A*31:51	Unconfirmed	A*31:91	Unconfirmed		
A*31:12	Confirmed	A*31:52	Unconfirmed	A*31:92	Unconfirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2017-April-13, release 3.28.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-A*31 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

HLA-A*31 SSP subtyping

Specificities and sizes of the PCR products of the 41+1 primer mixes used for
HLA-A*31 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*31 alleles ³	Other amplified HLA-A alleles
1	155 bp	800 bp	*31:01:02:01-31:07, 31:09-31:43, 31:45-31:86, 31:88, 31:90-31:135	*01:07, 01:226, 02:185, 02:601, 11:257, 23:21, 24:124, 26:19, 29:14, 30:12:01, 30:18, 30:55, 34:04, B*15:82, B*15:260, B*15:390, B*40:186:01, C*03:186:01, C*03:349
2⁴	80 bp 215 bp	800 bp	*31:67-31:68 *31:02, 31:07-31:08, 31:91, 31:109	*02:41, 02:80, 02:117, 02:289:01, 02:304, 02:454, 23:45, 24:62, 26:10, 32:28, 32:66, 33:32:01 *02:243:01-02:243:03, 24:82, 29:48, 33:08, 33:53
3	155 bp	800 bp	*31:03-31:04, 31:123	*02:309, 02:454, 03:01:19, 03:103:02, 25:19:01-25:19:02, 25:30, 26:43:01, 34:02:01, 34:02:03-34:04, 34:06-34:09, 34:13, 34:15, 66:06, 74:01:03
4	165 bp	1070 bp	*31:03-31:04, 31:06	*01:06, 01:200, 01:244, 02:114, 02:246, 02:279, 02:681, 03:01:30, 03:05:01-03:05:02, 03:42, 03:98, 03:105, 03:122, 11:24:01-11:25:02, 11:31, 11:35, 11:158, 23:53, 23:70, 29:01:01:01-29:01:02, 29:01:04-29:02:13, 29:02:15-29:04, 29:06-29:23, 29:25-29:27, 29:29-29:31, 29:34-29:76, 29:78N, 29:80-29:81, 29:83-29:86, 29:88-29:103, 29:105-29:112N, 30:26, 32:30:01-32:30:02, 32:32, 33:18:01-33:18:02, 34:02:01-34:04, 34:07-34:10N, 34:13, 34:15, 68:08:01-68:08:02, 68:63, 68:157, 68:168, 80:01:01:01-80:03
5⁴	120 bp 285 bp	800 bp	*31:26, 31:38 *31:03	*02:660, 03:184, 32:36 *01:143, 11:43, 29:66, 33:13, C*07:449
6	130 bp 165 bp	800 bp	*31:39, 31:54 *31:05	*33:07 *23:03:01, 24:21:03, 24:208, 29:03, 29:33, 32:13, 33:10
7	505 bp	1070 bp	*31:07-31:08, 31:10	*02:81, 02:87, 02:112, 02:124, 02:129, 02:571, 03:152, 03:219, 23:01:01:01-23:01:15,

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				23:01:17-23:01:21, 23:03:01-23:13, 23:14:02-23:26, 23:28-23:33, 23:35-23:37:02, 23:39-23:65, 23:67-23:68, 23:70-23:88, 24:13:01, 24:18, 24:24, 24:94, 24:188, 24:207:01, 24:228, 24:355, 24:392, 25:19:01-25:19:02, 25:30, 29:13, 32:01:01:01-32:01:05, 32:01:07-32:01:29, 32:03-32:09, 32:11Q-32:21, 32:23-32:70, 32:72-32:100, 32:102-32:106, 32:108-32:110
8	155 bp 220 bp	1070 bp	*31:24, 31:27 *31:09	*11:01:28, 11:01:77, 29:67, 32:26:02, 33:61, 33:127, 33:142
9⁴	75 bp	1070 bp	*31:01:02:01-31:02, 31:05, 31:07-31:61, 31:63-31:66, 31:70-31:119, 31:121-31:135	*02:24:02, 02:65, 02:152, 02:507, 23:03:01, 23:83, 24:21:03, 29:32, 32:01:01:01-32:01:06, 32:01:08-32:01:11, 32:01:13-32:01:27, 32:01:29-32:03, 32:05-32:27N, 32:29, 32:31, 32:33:01, 32:34-32:47, 32:49-32:65, 32:67-32:93, 32:95-32:100, 32:102-32:110, 33:01:01:01-33:01:04, 33:01:06-33:01:11, 33:03:01:01-33:03:18, 33:03:20-33:03:23, 33:03:25-33:03:26, 33:03:28-33:17, 33:20-33:31, 33:33-33:37, 33:39-33:146, 74:01:01-74:28, B*15:17:03
10^{4,5}	115 bp	1070 bp	*31:41	*02:24:02, 02:507, 24:21:03, 24:208, 29:33, 29:51, 29:80, 32:02, 32:06, 32:109, 33:94
11	160 bp 135 bp 210 bp	1070 bp	*31:11, 31:56 *31:26 *31:12, 31:60N	*02:660, 03:184, 32:36 *02:490N, 02:516N, 02:526, 03:269N, 32:89
12	245 bp	1070 bp	*31:01:02:01-31:06, 31:09, 31:11-31:20, 31:22-31:32, 31:34-31:78, 31:80-31:135	*02:243:01-02:243:03, 03:205, 11:43, 29:19, 29:39, 29:48, 33:01:01:01-33:01:11, 33:03:01:01-33:12, 33:14-33:16, 33:18:01-33:37, 33:39-33:47, 33:49-33:50, 33:52-33:68, 33:70-33:91, 33:93-33:108, 33:110-33:118, 33:120-33:146, 68:29
13⁴	85 bp	1070 bp	*31:13	*02:251
14⁶	150 bp 220 bp	800 bp	*31:24 *31:14N	*11:01:28, 11:01:77, 29:67, 32:26:02, 33:61
15	150 bp 225 bp	1070 bp	*31:25 *31:15	*32:26:01
16⁴	80 bp	1070 bp	*31:46	

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	165 bp		*31:16	*29:12, 29:92, 33:58
17	160 bp 235 bp	1070 bp	*31:25, 31:40 *31:17	*32:26:01
18	155 bp 200 bp	1070 bp	*31:43, 31:62 *31:18	*02:408, 02:590 *23:43, 33:132
19⁴	110 bp 185 bp	1070 bp	*31:19 *31:72	*02:380, 03:52, 30:109
20	325 bp	1070 bp	*31:20	
21⁴	75 bp 155 bp 180 bp 215 bp	1070 bp	*31:35 *31:43, 31:62 *31:21 *31:01:02:03N	*01:07 *02:408, 02:590 *01:07, 02:185, 02:601, 30:55
22⁴	80 bp 155 bp 190 bp	1070 bp	*31:71 *31:27, 31:55 *31:22	*02:327 *33:127, 33:142
23⁴	80 bp 165 bp 200 bp	1070 bp	*31:71 *31:40 *31:23	*02:327
24	150 bp 180 bp 220 bp	1070 bp	*31:55 *31:81 *31:28, 31:89, 31:115	*02:104 *03:205, 11:43, 33:125, 33:131 68:29
25⁵	135 bp 165 bp 245 bp	800 bp	*31:29 *31:56 *31:59	*02:507, 23:03:01, 23:83, 24:21:03, 24:208, 29:07, 32:89
26	130 bp	1070 bp	*31:30, 31:39, 31:97	*02:507, 29:28, 29:79, 32:10, 33:94, B*07:02:40, C*02:02:15, C*04:175
27⁴	185 bp 85 bp	800 bp	*31:72 *31:31, 31:67-31:68	*02:41, 02:80, 02:117, 02:289:01, 02:304, 02:454, 23:45, 24:62, 26:10, 32:28, 32:66, 33:32:01 *02:72, 02:275, 68:156
28⁴	500 bp 110 bp 175 bp 205 bp	1070 bp	*31:38 *31:32 *31:60N	*02:490N, 02:516N, 03:269N
29⁴	115 bp 180 bp 275 bp	1070 bp	*31:48 *31:44, 31:81 *31:36	*02:140, 26:99, 33:15 *02:104
30⁵	160 bp 185 bp 245 bp	800 bp	*31:34 *31:87 *31:33	
31⁴	75 bp 120 bp	1070 bp	*31:37 *31:41, 31:54	*02:24:02, 02:507, 24:21:03, 24:208, 29:33, 29:51, 29:80, 32:02, 32:06, 32:109, 33:07, 33:94
32	165 bp	1070 bp	*31:95	
33	180 bp	1070 bp	*31:96	
34	130 bp	1070 bp	*31:98	

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35	160 bp	1070 bp	*31:102	*24:282, 26:46
36	250 bp	1070 bp	*31:105	B*55:66
37	230 bp	1070 bp	*31:111	
38	305 bp	1070 bp	*31:119	
39 ⁴	110 bp	1070 bp	*31:125	*02:324, 02:426, 11:173
40	290 bp	1070 bp	*31:126N	*02:540N
41	240 bp	1070 bp	*31:131N	
42 ⁷	-	-	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-A*31 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 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 mixes 10, 25 and 30 may have tendencies of unspecific amplifications.

⁶Primer mix 14 may give rise to a lower yield of HLA-specific PCR product than the other HLA-A*31 primer mixes.

⁷Primer mix 42 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.

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Lot-specific Information
PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	155	80	155	165	120	130	505	155	75	115	135	245
		215			285	165		220		160	210	
Length of int. pos. control ¹	800	800	800	1070	800	800	1070	1070	1070	1070	1070	1070
5'-primer(s) ²	127 5'-ggg 3'	97 5'-TCA 3'	423 5'-gCT 3'	413 5'-CCg 3'	97 5'-TCA 3'	448 5'-CCT 3'	317 5'-gCT 3'	97 5'-TCA 3'	413 5'-CCA 3'	448 5'-CCT 3'	362 5'-gAA 3'	97 5'-TCA 3'
		414 5'-CAg 3'			445 5'-TCC 3'			448 5'-CCT 3'		706 5'-CgA 3'	375 5'-TgA 3'	
					467 5'-CTA 3'						445 5'-TCC 3'	
3'-primer(s) ³	238 5'-CCT 3'	270 5'-ACT 3'	538 5'-CAA 3'	539 5'-TCA 3'	341 5'-CgT 3'	530 5'-CCT 3'	538 5'-CAA 3'	214 5'-CCA 3'	448 5'-CAA 3'	524 5'-CAT 3'	538 5'-CAA 3'	299 5'-CCA 3'
	238 5'-CCT 3'	453 5'-TCT 3'			538 5'-CAA 3'	536 5'-ACg 3'		278 5'-ggC 3'		565 5'-CAg 3'		
	245 5'-ACg 3'					570 5'-CCg 3'		559 5'-CCg 3'		831 5'-TCC 3'		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15	16	17	18	19	20	21	22	23	24
Length of spec. PCR product	85	150	150	80	160	155	110	325	75	80	80	150
		220	225	165	235	200	185		155	155	165	180
									180	190	200	220
									215			
Length of int. pos. control ¹	1070	800	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070
5'-primer(s) ²	413 5'-CCA 3'	448 5'-CCT 3'	98 5'-CAC 3'	98 5'-CAC 3'	98 5'-CAC 3'	413 5'-CCA 3'	488 5'-ggT 3'	302 5'-ggA 3'	2 nd I 5'-CTC 3'	97 5'-TCA 3'	98 5'-CAC 3'	97 5'-TCA 3'
		629 5'-CAA 3'	448 5'-CCT 3'	769 5'-Agg 3'	448 5'-CCT 3'		635 5'-gCg 3'	302 5'-ggA 3'	98 5'-CTT 3'	652 5'-CTg 3'	652 5'-CTg 3'	652 5'-CTg 3'
									203 5'-gAA 3'			
									413 5'-CCA 3'			
3'-primer(s) ³	456 5'-TCg 3'	559 5'-CCg 3'	281 5'-AgC 3'	221 5'-ACA 3'	221 5'-ACC 3'	527 5'-CCg 3'	559 5'-CgT 3'	346 5'-AgC 3'	238 5'-CCT 3'	214 5'-CCA 3'	221 5'-ACC 3'	277 5'-ggT 3'
		808 5'-AgA 3'	559 5'-CCT 3'	808 5'-AgA 3'	292 5'-gTT 3'	571 5'-CCT 3'	777 5'-gCA 3'		527 5'-CCg 3'	245 5'-ACg 3'	691 5'-gCC 3'	282 5'-gAC 3'
					559 5'-CCT 3'					691 5'-gCC 3'	811 5'-CAT 3'	763 5'-CAA 3'
										763 5'-CAA 3'		791 5'-AgT 3'
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

101.430-12 – including *Taq* polymerase, IFU-01
101.430-12u – without *Taq* polymerase, IFU-02

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“Instructions for Use” (IFU)

Lot No.: **6G7**

Lot-specific Information

Well No.	25	26	27	28	29	30	31	32	33	34	35	36
Length of spec. PCR product	135 165	130 185	85 500	110 175	115 180	160 185	75 120	165	180	130	160	250
	245			205	275	245						
Length of int. pos. control ¹	800	1070	800	1070	1070	800	1070	1070	1070	1070	1070	1070
5'-primer(s) ²	355 5'-CCC 3'	448 5'-CCT 3'	235 5'-AgA 3'	375 5'-TgA 3'	97 5'-TCA 3'	97 5'-TCA 3'	448 5'-CCT 3'	878 5'-gCA 3'	386 5'-gTT 3'	148 5'-TgT 3'	495 5'-CAC 3'	28 5'-TCC 3'
	626 5'-CCT 3'	635 5'-gCg 3'	414 5'-CAg 3'	406 5'-gCT 3'	652 5'-CTg 3'	448 5'-CCT 3'						
	706 5'-CgA 3'			467 5'-CTA 3'								
3'-primer(s) ³	448 5'-CAA 3'	536 5'-ACg 3'	290 5'-CAA 3'	538 5'-CAA 3'	238 5'-CCC 3'	239 5'-gCT 3'	482 5'-TgC 3'	899 5'-ACg 3'	524 5'-CAC 3'	238 5'-CCT 3'	616 5'-CgT 3'	107 5'-ACT 3'
	831 5'-TCC 3'	539 5'-TCC 3'	453 5'-TCT 3'		331 5'-CTC 3'	299 5'-CCC 3'	524 5'-CAT 3'					
		777 5'-gCA 3'			727 5'-CCA 3'	568 5'-CTg 3'	530 5'-CCT 3'					
					791 5'-AgT 3'							
Well No.	25	26	27	28	29	30	31	32	33	34	35	36

Well No.	37	38	39	40	41
Length of spec. PCR product	230	305	110	290	240
Length of int. pos. control ¹	1070	1070	1070	1070	1070
5'-primer(s) ²	620 5'-g 3'	5 th I 5'-TAT 3'	763 5'-TCA 3'	369 5'-TAg 3'	97 5'-TCA 3'
3'-primer(s) ³	808 5'-AgA 3'	1040 5'-CCC 3'	831 5'-TCC 3'	616 5'-CgT 3'	295 5'-TCA 3'
Well No.	37	38	39	40	41

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.

101.430-12 – including *Taq* polymerase, IFU-01
 101.430-12u – without *Taq* polymerase, IFU-02

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Lot No.: **6G7**

Lot-specific Information

CELL LINE VALIDATION SHEET																				
HLA-A*31 SSP subtyping kit ²																				
				Well																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Prod No.:	201439101	201439102	201439103	201439104	201439105	201439106	201439107	201439108	201439109	201439110	201439111	201439112	201439113	201439114	201439115	201439116
	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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

101.430-12 – including *Taq* polymerase, IFU-01
101.430-12u – without *Taq* polymerase, IFU-02

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Lot No.: **6G7**

Lot-specific Information

CELL LINE VALIDATION SHEET																				
HLA-A*31 SSP subtyping kit ²																				
				Well																
				17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
				Prod. No.:	201439117	201439118	201439119	201439120	201782021	201439122	201439123	201560124	201439125	201439126	201439127	201439128	201439129	201439130	201439131	201782040
IHCW cell line ¹		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 CTV3953540	*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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

101.430-12 – including *Taq* polymerase, IFU-01
101.430-12u – without *Taq* polymerase, IFU-02

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Lot No.: **6G7**

Lot-specific Information

CELL LINE VALIDATION SHEET													
HLA-A*31 SSP subtyping kit ²													
				Well									
				33	34	35	36	37	38	39	40	41	
				Prod. No.:	201782033	201782034	201782035	201782036	201782037	201782038	201782039	201895040	201895041
IHCW cell line ¹		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	-	-	-	-	-	-	-	-	-

101.430-12 – including *Taq* polymerase, IFU-01
 101.430-12u – without *Taq* polymerase, IFU-02

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Lot No.: **6G7**

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 2, 5, 6, 8, 10, 11, 13 and 15 to 41 were available.

The specificities of the primers in primer solutions 2, 5, 6, 8, 10, 16, 21 to 27 and 31 were tested by separately adding one or two additional 5'-primers, respectively one, two or three additional 3'-primers. In primer solutions 13, 15, 17, 18, 20, 29, 30, 36, 38 and 41 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 11, 19, 28, 32 to 35, 37, 39 and 40 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solution 5, 10, 16, 21 and 25 to 27 one or two 5'-primers were not possible to test, and in primer solutions 1, 6, 8, 10, 16, 21 to 24, 26 and 31 one, two or three 3'-primers were not possible to test. Additional primers in primer solution 14 were tested by separately adding one 5'-primer respectively one 3'-primer.

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