

101.401.48/12 – including *Taq* pol., IFU-01
101.401.48u/12u – without *Taq* pol., IFU-02

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

Lot No.: **78Y**

Lot-specific information

Olerup SSP® HLA-A low resolution

Product number:	101.401-48/12 – including <i>Taq</i> polymerase 101.401-48u/12u – without <i>Taq</i> polymerase
Lot number:	78Y
Expiry date:	2018-February-01
Number of tests:	48 tests – Product No. 101.401-48/48u 12 tests – Product No. 101.401-12/12u
Number of wells per test:	31 +1
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

This Product Description is only valid for Lot No. 78Y.

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 LOW RESOLUTION LOT (42X)

The format of the Product Insert and Worksheet have been changed.

The **HLA-A low resolution** specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP®* HLA-A low resolution lot was made (**Lot No. 42X**). The kit design is based on IMGT/HLA database 3.20.0.

As of lot series V, the Specificity Table is included in the lot-specific Product Insert, and the Interpretation Table is included in the Worksheet.

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

Well	5'-primer	3'-primer	rationale
9	-	Added	3'-primer added for the A*25:19:02 allele.
16	-	Added	3'-primer added for the A*31:89 allele.
21	Exchanged	Added	5'-primer exchanged and 3'-primer added for the A*23:66 allele.
22	Added	-	5'-primer added for the A*68:11N allele.
27	Added	-	5'-primer for increased yield.
29	Added	Added	Primer pair added for the A*24:09N allele.
31	Added	Added	Primer pair added for the A*24:11N allele.

Change in revision R01 compared to R00:

1. The footnote numbers for primer mix 21 have been corrected in the Specificity Table.

Change in revision R02 compared to R01:

1. Primer mix 29 does not amplify the A*24:09N allele. This has been corrected in the Interpretation and Specificity Tables. Thus, this lot of the HLA-A low subtyping kit cannot distinguish the A*24:02:01:01-24:05, 24:08, 24:10:01-24:10:02, 24:13:02, 24:17, 24:20-24:21:02, 24:23, 24:25-24:43, 24:45N-24:50, 24:54-24:56, 24:58-24:63, 24:66-24:70, 24:72-24:81, 24:83N-24:91, 24:93, 24:95-24:107, 24:109-24:111, 24:113, 24:115-24:130, 24:132N-24:137, 24:139-24:166, 24:168-24:187, 24:189-24:203, 24:205-24:206, 24:208-24:210, 24:212, 24:214-24:221, 24:223-24:227, 24:229-24:242, 24:244-24:287, 24:289, 24:292-24:293, 24:295, 24:297-24:303N, 24:305 alleles and the A*24:09N allele.

Change in revision R03 compared to R02:

1. Primer mix 29 weakly amplifies the A*24:09N allele. This has been corrected in the Interpretation and Specificity Tables.

Change in revision R04 compared to R03:

1. Primer mix 21 may generate a false positive band of about 560 base pairs. This band should be disregarded when interpreting HLA-A low resolution typings. A footnote has been added in the Specificity Table.

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Well **32** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup SSP*® HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

HLA-specific PCR product sizes range from 75 to 200 base pairs.
The PCR product generated by the positive control primer pair is 430 base pairs.

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

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

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

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PRODUCT DESCRIPTION

HLA-A low resolution

CONTENT

The primer set contains 5'- and 3'-primers for grouping the HLA-A*01:01 to A*80:03 alleles into the corresponding serological groups A1 to A80.

PLATE LAYOUT

Each test consists of 32 PCR reactions in a 32 well PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	NC

The 32 well PCR plate is marked with 'HLA-A low' in silver/gray ink.

Well No. 1 is marked with the Lot No. '78Y'.

Wells 1 to 31 – HLA-A low resolution primers.

Well 32 – Negative Control.

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

Only HLA-A alleles will be amplified by the 32 wells of the HLA-A low resolution primer set, except that a few HLA-B and HLA-C alleles will be amplified by primer mixes 4 to 6, 9 and 11.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A alleles, i.e. **A*01:01 to A*80:03**, recognized by the HLA Nomenclature Committee in April 2015^{1,2} will be amplified by the primers in the HLA-A low resolution primer set³. The HLA-A alleles will be grouped into their corresponding serological specificities.

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

Alleles	Primer mix
A*24:14-24:15, 24:51-24:53, 24:57, 24:64, 24:114, 24:222N, 24:291, 24:296, C*04:01:03	5

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¹HLA-A alleles listed on the IMGT/HLA web page 2015-April-17, release 3.20.0, www.ebi.ac.uk/imgt/hla.

²Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page <http://hla.alleles.org/alleles/deleted.html>.

³The A*23:14:01-23:14:02 and the A*24:71 alleles will give rise to identical amplification patterns. These alleles can be separated by the respective high resolution SSP primer sets. The A*23:57 and 23:66 and the A*24:94, 24:138, 24:188 and 24:228 alleles will give rise to identical amplifications patterns. These alleles can be separated by the respective high resolution SSP primer sets.

The A*31:08 and 33:53 alleles will give rise to identical amplifications. These alleles can be separated by the respective high resolution SSP primer sets.

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Lot-specific information
SPECIFICITY TABLE

HLA-A low resolution primer set

Specificities and sizes of the PCR products of the 31+1 primer mixes used for HLA-A low resolution SSP typing

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	HLA-A serology ³	Amplified HLA-A alleles ^{4,5}
1⁶	120 bp, 140 bp	800 bp	A1, A36	*01:01:01:01-01:01:31, 01:01:33-01:01:59, 01:01:61-01:04N, 01:06-01:33, 01:35-01:68, 01:70-01:142, 01:144-01:170, 01:172-01:175, 01:178N-01:179N, 36:01-36:05
2¹²	175 bp, 215 bp, 255 bp, 365 bp, 415 bp	800 bp	A2, A19, A28, A203, A210	*02:01:01:01-02:01:15, 02:01:17-02:01:19, 02:01:21-02:01:81, 02:01:83-02:22:02, 02:24:01-02:35:01, 02:35:03-02:47, 02:49-02:77, 02:78 ^w , 02:79:01-02:97:02, 02:99, 02:101:01-02:128, 02:130-02:570, 02:572-02:573
3⁷	235 bp	1070 bp	A3, A32/A3, A36	*03:01:01:01-03:01:29, 03:01:31-03:01:34, 03:01:36-03:01:47, 03:01:51-03:04:03, 03:06-03:09, 03:11N-03:17:02, 03:19-03:39, 03:41, 03:43-03:74, 03:76-03:94, 03:96-03:97, 03:99-03:104, 03:106-03:121, 03:123:01-03:134, 03:136-03:166, 03:168N-03:176, 03:178N-03:186, 03:188-03:203, 03:205-03:207, 03:209-03:214, 03:216-03:219, 03:221, 11:130, 30:89, 32:04, 36:02
4	190 bp	800 bp	A1, A3, A11, A30, A36, A68	*01:01:01:01-01:01:22, 01:01:24-01:01:47, 01:01:49-01:01:64, 01:01:67-01:04N, 01:06-01:07, 01:09-01:11N, 01:13, 01:16N-01:18N, 01:20-01:29, 01:31N-01:33, 01:35-01:78, 01:80-01:98, 01:100-01:144, 01:146, 01:148, 01:150-01:158, 01:160N-01:166, 01:168-01:175, 02:78, 02:169, 03:12, 03:18, 03:88, 03:135, 11:01:01:01-11:27, 11:29-11:52Q, 11:54-11:215N, 26:19, 26:72, 29:67, 30:08, 32:64, 36:04, 68:13:01, 68:66, 74:19, C*12:131
5¹²	160 bp, 335 bp, 505 bp	1070 bp	A3, A9, A23, A24, A2403, A29, A31, A32	*03:15, 03:19, 03:30, 03:152, 11:139, 23:01:01-23:69, 24:02:01:01-24:11N, 24:13:01-24:15, 24:17-24:64, 24:66-24:210, 24:212-24:303N, 24:305, 29:07, 29:49, 31:08, 31:29, 32:05, 33:19, 33:53, C*04:01:03
6^{8,14}	135 bp, 200 bp	800 bp	A9, A23, A24, A29, A80	*11:166, 23:01:01-23:56, 23:58-23:65, 23:67-23:69, 24:24, 24:71, 29:07, 29:49, 31:29, 31:85, 32:72, 80:01:01:01-80:03, B*18:27
7¹²	175 bp, 205 bp	1070 bp	A2, A9, A23, A24, A2403, A26, A33	*02:17:01 ^w -02:17:03 ^w , 11:139, 23:14:01-23:14:02, 24:02:01:01-24:11N, 24:13:01-24:13:02, 24:17-24:50, 24:54-24:56, 24:58-24:63, 24:66-24:91, 24:93, 24:95-24:113, 24:115-24:137, 24:139-24:187, 24:189-24:210, 24:212-24:221, 24:223-24:227, 24:229-24:290, 24:292-24:295, 24:297-24:303N, 24:305, 26:16, 33:19, 68:45, 68:117

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8¹²	165 bp, 200 bp	800 bp	A2/A28, A3, A10, A11, A25, A26, A32, A34, A66, A68, A69	*01:51, 02:55, 02:527, 03:24, 03:50, 11:10, 11:183, 11:191, 25:01:01-25:16, 25:18-25:31, 26:01:01:01-26:06, 26:08-26:15, 26:17-26:18, 26:20-26:43:02, 26:45-26:63, 26:65-26:71N, 26:73-26:88, 26:90-26:91, 26:93-26:111, 29:28, 32:15, 33:51, 34:01:01-34:12, 66:01:01-66:22, 68:01:01:01-68:132, 69:01-69:03
9^{6,9}	80 bp	800 bp	A3, A25, A32	*25:01:01-25:31, 32:01:01-32:02, 32:04, 32:06- 32:37, 32:39-32:59, 32:61-32:70, 32:72, B*07:81, B*08:52, B*18:67, B*38:41, B*51:185, B*53:05, B*53:16, B*53:33
10^{6,12,14}	80 bp, 240 bp	800 bp	A10, A26, A43	*01:43, 01:51, 11:17, 11:40, 26:01:01:01- 26:02:02, 26:04, 26:07:01-26:20, 26:22-26:29, 26:31-26:43:02, 26:45-26:77, 26:79-26:91, 26:93- 26:110, 33:13, 33:48, 43:01, 68:84
11^{6,12}	80 bp, 175 bp, 500 bp	1070 bp	A1, A9, A11, A26, A31, A34, A66	*01:13, 01:28, 01:136, 03:63, 03:88, 11:01:01:01- 11:27, 11:29-11:52Q, 11:54-11:215N, 24:19, 24:44, 26:03:01, 26:06, 26:21, 26:78, 26:92, 26:111, 34:01:01-34:08, 34:10N-34:12, 66:01:01- 66:01:03, 66:04-66:11, 66:13-66:14, 66:17-66:20, 66:22, 69:02, 80:02, C*07:404
12⁶	125 bp, 185 bp	800 bp	A3, A10, A25, A26, A31, A34, A43, A66	*02:309, 02:454, 03:01:19, 11:11, 25:05-25:06, 26:09, 26:54, 26:91, 31:03-31:04, 34:01:01-34:12, 43:01, 66:02-66:03, 66:16, 66:21, 68:130
13	175 bp, 225 bp	1070 bp	A1, A2, A3, A10, A25, A26, A34, A43, A66	*01:01:56 ^w , 01:13, 02:34-02:35:03, 02:56:01- 02:56:02, 02:62, 02:103, 02:135, 03:01:01:01- 03:01:22, 03:01:24-03:07, 03:09-03:11N, 03:13- 03:31, 03:33-03:35, 03:37-03:40, 03:42-03:56, 03:58, 03:60-03:71, 03:73-03:87, 03:90-03:106, 03:109-03:110, 03:112-03:141, 03:143-03:151, 03:153-03:171, 03:174-03:175, 03:177, 03:179- 03:197N, 03:200Q-03:202, 03:204, 03:206- 03:210, 03:212-03:218, 03:221, 11:199:01, 25:01:01-25:01:08, 25:02-25:05, 25:07-25:31, 26:01:01:01-26:01:20, 26:01:22-26:01:38, 26:02:01 ^w -26:02:02 ^w , 26:03:01, 26:05-26:08, 26:10-26:28, 26:29 ^w , 26:30-26:33, 26:35-26:43:02, 26:45-26:48, 26:49 ^w , 26:50-26:72, 26:74-26:77, 26:79-26:90, 26:92-26:111, 30:55, 34:08, 43:01, 66:01:01, 66:01:03, 66:04-66:09, 66:10 ^w , 66:11- 66:15, 66:17-66:20, 66:22, 68:71, 74:13
14^{6,12,14}	80 bp, 115 bp, 200 bp, 240 bp, 460 bp	1070 bp	A26, A29, A31, A34	*02:237, 02:309, 02:454, 03:95, 26:19, 26:22, 29:01:01:01-29:78N, 31:03-31:04, 32:42, 34:04, 66:09
15^{6,12,13,15}	90 bp, 135 bp, 205 bp	1070 bp	A1, A30	*01:13, 01:28, 03:43, 03:82, 03:186, 11:113, 11:162, 30:01:01-30:04:02, 30:06-30:20, 30:22- 30:94, 31:35
16	240 bp, 370 bp, 400 bp	1070 bp	A24, A31, A32	*02:237, 03:95, 29:14, 31:01:02:01-31:94, 32:05, 33:53, 74:13

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17	140 bp, 180 bp	1070 bp	A32, A36, A74	*01:95, 03:43, 03:82, 03:186, 24:243, 29:13, 29:39, 30:89, 31:35, 32:01:01-32:01:17, 32:01:19-32:03, 32:05-32:71, 74:07
18	200 bp, 390 bp	1070 bp	A32, A33, A68, A74	*02:243:01-02:243:02, 29:48, 32:15, 33:01:01-33:01:07, 33:03:01-33:37, 33:39-33:52, 33:54-33:97, 68:29, 74:04, 74:21
19	340 bp, 375 bp	800 bp	A2, A19, A68, A74	*01:121, 02:65, 02:407, 02:449, 32:62, 68:25, 74:01-74:24
20¹⁴	210 bp, 240 bp	800 bp	A2, A25, A28, A68	*02:34-02:35:03, 02:46, 02:48, 02:56:01-02:56:02, 02:62, 02:70, 02:78, 02:103, 02:129, 23:01:13, 25:05, 26:54, 34:02:04, 68:01:01:01-68:132
21^{14,17}	200 bp, 240 bp, 375 bp, 545 bp, 560 bp	800 bp	A2, A23, A24, A26, A28, A66, A68, A69	*02:17:01-02:17:03, 02:55, 02:108, 02:110, 02:243:01-02:243:02, 02:268, 02:300, 02:303, 23:02, 23:57, 23:66, 24:06, 24:13:01, 24:22, 24:82, 24:94, 24:138, 24:167, 24:188, 24:207, 24:228, 25:30, 26:22, 33:22, 66:06, 66:09, 68:08:02, 68:29, 68:105, 69:01-69:03
22^{6,11}	85 bp, 240 bp, 400 bp	800 bp	A2, A23, A34, A36	*02:34-02:35:03, 02:46, 02:48, 02:56:01-02:56:02, 02:62, 02:70, 02:78, 02:103, 02:129, 03:187, 11:155, 23:01:13, 31:62, 34:02:04, 36:01-36:05, 68:11N
23^{6,14}	75 bp, 160 bp, 240 bp, 495 bp	800 bp	A2/A28, A24/A3, A26, A36, A68, A80	*02:55, 02:237, 03:41, 03:63, 03:75, 03:88, 03:95, 03:177, 11:130, 24:18, 24:204, 24:213, 26:03:01, 26:05-26:06, 26:21, 26:30, 26:78, 26:111, 33:24, 36:02, 68:05, 68:15, 68:20, 68:109, 80:01:01:01-80:01:01:02, 80:03
24	360 bp	1070 bp	A3, A10, A26, A31, A66	*02:135, 02:309, 02:454, 03:01:19, 25:13, 26:30, 26:65, 31:04, 34:09, 66:02-66:03, 66:12, 66:16, 66:21
25	190 bp, 400 bp	1070 bp	A1, A29, A30, A31, A32, A33, A74	*29:01:01:01-29:29, 29:31-29:78N, 30:01:01-30:01:07, 30:01:09-30:04:02, 30:06-30:11:02, 30:13-30:17, 30:19-30:20, 30:22-30:30, 30:32-30:40, 30:42-30:54, 30:56-30:59N, 30:61-30:94, 31:01:02:01-31:08, 31:10-31:27, 31:29-31:94, 32:01:01-32:03, 32:05-32:23, 32:25-32:48N, 32:50-32:72, 33:01:01-33:01:07, 33:03:01-33:37, 33:39-33:97, 74:01-74:24
26	195 bp, 225 bp	800 bp	A1, A36, A43	*01:01:01:01-01:02, 01:04N, 01:07-01:11N, 01:13, 01:16N-01:18N, 01:21-01:33, 01:35-01:52N, 01:54-01:62, 01:64, 01:67:01-01:72, 01:74-01:97, 01:99-01:126, 01:128-01:129, 01:131-01:135, 01:137-01:175, 01:178N-01:179N, 03:18, 03:135, 11:11, 11:94, 11:112, 11:211, 36:04, 43:01, 68:130
27¹³	205 bp	1070 bp	A1, A3, A11, A30, A31, A32, A34	*01:12, 01:19, 01:21, 01:126, 02:156, 02:338, 03:01:01:01-03:01:05, 03:01:07-03:01:13, 03:01:15-03:01:46, 03:01:48, 03:01:50-03:01:51, 03:01:53-03:17:02, 03:19-03:49, 03:51-03:53, 03:55-03:63, 03:65, 03:67-03:74, 03:76-03:94, 03:96-03:126, 03:128-03:134, 03:136-03:139, 03:141-03:152, 03:154-03:164, 03:166-03:176, 03:178N-03:186, 03:188-03:199, 03:201-03:203, 03:205-03:214, 03:216-03:219, 03:221, 11:03, 11:20, 11:25, 11:31, 11:35, 11:60, 11:158, 11:175, 11:183, 11:209, 24:92, 30:04:01-30:04:02, 30:06,

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				30:09, 30:17, 30:29, 30:46, 30:77, 30:89-30:90, 31:03-31:04, 32:04, 33:49, 34:02:01-34:04, 34:07-34:10N, 68:103:01-68:103:02, 74:23
28^{6,13}	100 bp, 520 bp	1070 bp	A1, A2, A3, A9, A11, A23, A24, A28, A29, A30, A66, A68, A69	*01:13, 01:17, 02:34-02:35:03, 02:56:01-02:56:02, 02:62, 02:78, 02:103, 03:01:01:01-03:07, 03:09-03:28, 03:30-03:31, 03:33, 03:35, 03:37-03:40, 03:42-03:61, 03:64-03:87, 03:90-03:106, 03:109-03:151, 03:153-03:171, 03:173-03:175, 03:177-03:197N, 03:199-03:219, 03:221, 11:12, 11:155, 23:13, 24:07:01-24:07:02, 24:24, 24:108, 24:112, 24:131, 24:288, 24:290, 24:294Q, 29:01:01:01-29:18, 29:21-29:29, 29:31-29:33, 29:35-29:47, 29:49-29:65, 29:67-29:73, 29:75-29:78N, 30:01:01-30:01:10, 30:08, 30:11:01-30:11:02, 30:14L-30:20, 30:23-30:26, 30:30, 30:35-30:43, 30:48-30:49, 30:52-30:56, 30:58-30:60, 30:62-30:63, 30:65, 30:71-30:75, 30:78N-30:79, 30:81-30:83, 30:86-30:89, 30:91-30:94, 31:89, 32:17, 34:09, 66:02, 66:12, 66:16, 66:21, 68:01:01:01-68:01:17, 68:01:19-68:02:09, 68:06-68:14, 68:16-68:19, 68:21:01-68:30, 68:32-68:39, 68:41-68:71, 68:73-68:84, 68:86-68:89, 68:91-68:92, 68:94N-68:96, 68:98-68:108, 68:110-68:132, 69:01, 69:03, 74:13
29^{6,15}	105 bp, 260 bp	800 bp	A3, A11, A26, A30, A68, A74	*01:134, 03:09, 03:108, 03:172, 03:198, 11:06, 11:18, 24:09N ^w , 26:03:01, 26:06, 26:21, 26:30, 26:78, 26:92, 26:111, 29:19, 29:48, 30:13, 30:16, 30:44, 30:46, 33:24, 68:05, 68:15, 68:20, 74:06, 74:21
30¹⁰	180 bp	1070 bp	A10, A25, A26, A43, A66	*01:01:56, 02:135, 25:01:01-25:01:08, 25:02-25:05, 25:07-25:17, 25:19:01-25:31, 26:01:01:01-26:01:20, 26:01:22-26:03:01, 26:05-26:08, 26:10-26:33, 26:35-26:43:02, 26:45-26:72, 26:74-26:77, 26:79-26:90, 26:92-26:102, 26:104-26:111, 43:01, 66:01:01, 66:01:03, 66:04-66:15, 66:17-66:20, 66:22
31⁶	110 bp, 195 bp, 465 bp	1070 bp	A34	*01:04N, 03:21N, 11:21N, 11:191, 23:07N, 24:11N, 34:01:01-34:02:04, 34:04-34:05, 34:07-34:12
32¹⁶	-	-	-	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 low resolution SSP typings.

When the primers in a primer mix can give rise to HLA-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits, the alleles listed are specified according to amplicon length.

Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings. Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal

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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.

³The serological reactivity of all HLA-A alleles is not known. In this table we use the expert-assigned serological grouping in Tissue Antigens (2009) 73:95-170 and the serological grouping of the sequence-defined allele.

⁴For 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.

⁵Only HLA-A alleles will be amplified by the 32 wells of the HLA-A low resolution primer set, except that a few HLA-B and HLA-C alleles will be amplified by primer mixes 4 to 6, 9 and 11.

⁶HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁷Primer mix 3 may faintly amplify the A*30:04:01-30:04:02, 30:06, 30:17 and 30:29 alleles.

⁸Primer mix 6 may give rise to a lower yield of A*23 alleles than the other A low primer mixes.

⁹Primer mix 9 may weakly amplify the A*34 alleles.

¹⁰Primer mix 30 may generate a false positive band of about 500 base pairs. This band should be disregarded when interpreting HLA-A low resolution typings.

¹¹Primer mix 22 might faintly amplify most A*11 alleles.

¹²The primer pairs in wells 2, 5, 7, 8, 10, 11, 14 and 15 will in many samples give rise to two or three HLA-specific PCR fragments.

¹³Primer mixes 15, 27 and 28 may give rise to a lower yield of HLA-specific PCR product than the other HLA-A low primer mixes.

¹⁴Primer mixes 6, 10, 14, 20, 21 and 23 have a tendency to giving rise to primer oligomer formation.

¹⁵Primer mixes 15 and 29 may have tendencies of unspecific amplifications.

¹⁶Primer mix 32 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by the control primer pairs matching the human growth hormone gene. HLA-specific PCR product sizes range from 75 to 200 base pairs and the PCR product generated by the HGH positive control primer pair is 430 base pairs.

¹⁷Primer mix 21 may generate a false positive band of about 560 base pairs. This band should be disregarded when interpreting HLA-A low resolution typings.

‘w’, might be weakly amplified.

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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	120	175	235	190	160	135	175	165	80	80	80	125
	140	215			335	200	205	200		240	175	185
		255			505						500	
		365										
		415										
Length of int. pos. control ¹	800	800	1070	800	1070	800	1070	800	800	800	1070	800
5'-primer(s) ²	98	48	363	98	144	176	98	98	261	98	301	103
	5'-CTT 3'	5'-gCT 3'	5'-ATA 3'	5'-CTA 3'	5'-gCC 3'	5'-gCA 3'	5'-CTC 3'	5'-CTA 3'	5'-AAC 3'	5'-CTA 3'	5'-Cgg 3'	5'-CCCT 3'
	103	78		413	317	368	368	102	266	261	302	415
	5'-CCT 3'	5'-TCT 3'		5'-CCg 3'	5'-gCT 3'	5'-gTT 3'	5'-gTT 3'	5'-ACA 3'	5'-ACg 3'	5'-AAC 3'	5'-ggA 3'	5'-ggT 3'
	123	106						413			385	423
	5'-AgT 3'	5'-CCA 3'						5'-CCg 3'			5'-ggC 3'	5'-gCT 3'
3'-primer(s) ³	203	240	555	256	265	270	259	259	302	299	341	257
	5'-TCT 3'	5'-ggA 3'	5'-gCA 3'	5'-CTg 3'	5'-CCC 3'	5'-ACA 3'	5'-gTT 3'	5'-gTT 3'	5'-ggC 3'	5'-TCg 3'	5'-CgT 3'	5'-gCA 3'
		292	555	559	368	521	502	259	303		521	506
		5'-gTg 3'	5'-CCA 3'	5'-CCg 3'	5'-CAA 3'	5'-ggg 3'	5'-CTT 3'	5'-gTT 3'	5'-AgA 3'		5'-ggg 3'	5'-TgT 3'
						534	539	538				559
						5'-CgT 3'	5'-TCT 3'	5'-CCA 3'				5'-CTC 3'
												559
												559
												559
												559
												559
												559
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	175	80	90	240	140	200	340	210	200	85	75	360
	225	115	135	370	180	390	375	240	240	240	160	
		200	205	400					375	400	240	
		240							545		495	
		460							560			
Length of int. pos. control ¹	1070	1070	1070	1070	1070	1070	800	800	800	800	800	1070
5'-primer(s) ²	98	98	203	41	180	41	302	78	28	47	176	341
	5'-CTT 3'	5'-CAC 3'	5'-gAA 3'	5'-CTT 3'	5'-TTT 3'	5'-CTT 3'	5'-ggA 3'	5'-TCT 3'	5'-TCg 3'	5'-g.T 3'	5'-gCA 3'	5'-ggC 3'
	423	219	362	355	203	98	302	106	261	78	261	
	5'-gCT 3'	5'-gCA 3'	5'-ggT 3'	5'-CCg 3'	5'-gAA 3'	5'-CAC 3'	5'-ggA 3'	5'-CCA 3'	5'-AAC 3'	5'-TCT 3'	5'-AAC 3'	
		238	363		418		341	2 nd	368	527	341	
		5'-AgA 3'	5'-ATA 3'		5'-AgC 3'		5'-ggA 3'	5'-CCT 3'	5'-gTT 3'	5'-TgC 3'	5'-ggA 3'	
		355	363								355	
		5'-CCg 3'	5'-ATA 3'								5'-CCC 3'	
			363								362	
			5'-ATA 3'								5'-gAg 3'	
			369								362	
			5'-TAC 3'								5'-gAg 3'	
3'-primer(s) ³	282	180	299	238	290	256	397	265	97	265	292	418
	5'-gAC 3'	5'-TCA 3'	5'-CCA 3'	5'-CCT 3'	5'-CAA 3'	5'-CCC 3'	5'-gAg 3'	5'-CCC 3'	5'-ggT 3'	5'-CCC 3'	5'-gTg 3'	5'-gTC 3'
	282	257	411	238	317	256		282	355	282	292	
	5'-gAC 3'	5'-gCA 3'	5'-TCA 3'	5'-CCT 3'	5'-ggA 3'	5'-CTC 3'		5'-gAC 3'	5'-gAC 3'	5'-gAC 3'	5'-gTT 3'	
	559	418	526	243	555	259		282	524	282	299	
	5'-CCC 3'	5'-gTC 3'	5'-CCA 3'	5'-TCA 3'	5'-CCA 3'	5'-gTT 3'		5'-gAC 3'	5'-CAT 3'	5'-gAC 3'	5'-TCT 3'	
	559	555		265				502	538	570	555	
	5'-CCg 3'	5'-CCA 3'		5'-CCC 3'				5'-CTT 3'	5'-CCA 3'	5'-CAC 3'	5'-CCA 3'	
				282				506				
				5'-gAC 3'				5'-TgT 3'				
				555								
				5'-CCA 3'								
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

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Well No.	25	26	27	28	29	30	31
Length of spec. PCR product	190	195	205	100	105	180	110
	400	225		520	260		195
							465
Length of int. pos. control ¹	1070	800	1070	1070	800	1070	1070
5'-primer(s) ²	41	103	363	282	74	418	270
	5'-CTT 3'	5'-CCT 3'	5'-ATA 3'	5'-CAg 3'	5'-C 3'	5'-AgA 3'	5'-AAA 3'
	123	363		282	78	423	363
	5'-AgT 3'	5'-ATA 3'		5'-CAg 3'	5'-TCC 3'	5'-gCT 3'	5'-ATA 3'
					678		3 rd I
					5'-AgA 3'		5'-A 3'
3'-primer(s) ³	270	257	527	341	292	559	341
	5'-ACT 3'	5'-gCA 3'	5'-CCA 3'	5'-Cgg 3'	5'-gTg 3'	5'-CCg 3'	5'-CgT 3'
	270	545	527	521	742	559	517
	5'-ACA 3'	5'-AgA 3'	5'-CCT 3'	5'-ggA 3'	5'-CTA 3'	5'-CCg 3'	5'-CgT 3'
			527				621
			5'-CCT 3'				5'-ggg 3'
Well No.	25	26	27	28	29	30	31

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

²The nucleotide position matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

³The nucleotide position matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

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Lot-specific information

CELL LINE VALIDATION SHEET																				
HLA-A low resolution primer set ²																				
				Well																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Prod No.:	201556301	201553402	201556303	201553404	201553405	201556306	201553407	201553408	201556309	201553410	201553411	201553412	201553413	201553414	201553415	201553416
	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	-	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-
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	+	-	+	+	-	-	W	-	-	-	-	-	+	-	-	-	-
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	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-

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HLA-A low resolution primer set ²																		
				Well														
				17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
				Prod No.:														
				201553417	201553418	201553419	201553420	201556321	201556322	201553423	201552924	201556325	201556326	201556327	201556328	201556329	201556330	201556331
	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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

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

101.401.48/12 – including *Taq* pol., IFU-01
101.401.48u/12u – without *Taq* pol., IFU-02

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Lot No.: 78Y

Lot-specific information

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.

Additional 5'- and 3'-primers in primer solutions 4, 5, 7, 8, 12, 14 to 17, 20 to 23, 26 and 31 were tested by separately adding one 3'-primer, respectively one 5'-primer. Additional 5'-primers in primer solution 1, 19 and 29 were tested by separately adding one 3'-primer. Additional 3'-primers in primer solutions 18 and 27 were tested by separately adding one 5'-primer.

In primer solutions 2, 11, 12, 15, 22 and 29 one or two 5'-primers were not possible to test, and in primer solutions 3, 6, 8, 9, 12, 13, 16, 18 and 29 one 3'-primer was not possible to test.

101.401.48/12 – including *Taq* pol., IFU-01
101.401.48u/12u – without *Taq* pol., IFU-02

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Lot No.: **78Y**

Lot-specific information

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