

101.572-12 – including *Taq* pol., IFU-01
101.572-12u – without *Taq* pol., IFU-02

Visit <https://labproducts.caredx.com> for
“Instructions for Use” (IFU)

Lot No.: **5G6**

Lot-specific information

Olerup SSP® HLA-B*57:01

Product number:	101.572-12 – including <i>Taq</i> polymerase 101.572-12u – without <i>Taq</i> polymerase
Lot number:	5G6
Expiry date:	2021-03-01
Number of tests:	12
Number of wells per test:	23+1
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

This Product Description is only valid for Lot No. 5G6.

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-B*57:01 LOT (5E0).

Positive and negative control DNAs are included in the kit.

The format of the Worksheet has been changed

One well has been added to HLA-B*57:01, well **24**.

The HLA-B*57:01 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP®* HLA-B*57:01 lot was made (**Lot No. 5E0**). The kit design is based on IMGT/HLA database 3.31.0.

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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
4	Added	-	5'-primer added for the B*57:91 allele.
8	Removed, added	Exchanged	Excess 5'-primer removed, 3'-primer exchanged, 5'-primer added for the B*57:87 allele.
13	Added	-	5'-primer added for the B*57:90 allele.
14	Removed, added	-	Excess 5'-primer removed for decreased tendency of oligomer formation, 5'-primer added for the B*57:87 allele.
18	Added	-	5'-primer added for the B*57:86 allele.
19	-	Added	3'-primer added for the B*57:92 allele.
20	Exchanged	Added	5'-primer exchanged, 3'-primer added for the B*57:44 allele.
22	Removed, added	Removed, added	Primer pair for the B*57:67:02 removed for improved allelic resolution, primer pair added for the B*57:85 allele.
23	Added	Added	Negative control moved to well 24, primer pair added for the B*57:59 allele.
24	-	-	Negative control added from well 23.

Change in revision R01 compared to R00:

1. Primer mix 22 has a tendency for unspecific amplification. A footnote has been added in the Specificity Table.

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Well **24** 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-B*57:01 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*57:01:01 to 57:01:24 alleles.

The primer solutions consist of specific primer mixes, i.e. group-specific primers as well as a **control primer pair** matching non-allelic sequences.

Positive and negative control DNAs are included in the kit:

DNA 1; a B*57-positive DNA as a positive control, **IHW 9052, DBB, B*57:01.**

DNA 2; a B*58-positive DNA as a negative control, **IHW 9285, WT49, B*58:01.**

(A B*58:01-positive DNA was chosen as negative control, as this is most similar to the B*57 group of alleles in the primer matching regions.)

The kit contains enough control DNAs to perform 1 test set up of positive and negative control. If more than 1 test set up per kit are run other positive and negative DNA samples can be used as controls (e.g. positive and negative samples from previous tests).

Legal notice: The DNA in this kit is included under licensing agreement between Olerup SSP AB and Public Health England.

PLATE LAYOUT

Each test consists of 24 PCR reactions in a 24 well cut PCR plate.

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

The 24 well cut PCR plate is marked with 'B*57:01' in silver/gray ink.

Well No. 1 is marked with the Lot No. '5G6'.

Wells 1 to 23 – HLA-B*57:01 primers.

Well 24 – Negative Control (NC).

A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.

The PCR plates are heat-sealed with a PCR-compatible foil.

Please note: When removing each 24 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Due to the sharing of sequence motifs between HLA-B alleles some non-HLA-B*57 alleles will be amplified by primer mixes. For further details see Specificity Table.

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UNIQUELY IDENTIFIED ALLELES

HLA-B*57:01 will give rise to a unique amplification pattern by the primers in the HLA-B*57:01 kit^{1,2,3}.

¹HLA-B alleles listed on the IMGT/HLA web page 2018-January-19, release 3.31.0,
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>.

³This lot of the B*57:01 kit cannot distinguish the B*57:01 alleles and the B*57:67:02 allele.

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SPECIFICITY TABLE

HLA-B*57:01 SSP subtyping

Specificities and sizes of the PCR products of the 23+1 primer mixes used for HLA-B*57:01 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*57:01 alleles ³	Other amplified Class I alleles
1⁴	90 bp	800 bp	*57:01:01:01-57:01:24	*57:02:01-57:04:01, 57:05-57:15, 57:17-57:19, 57:21-57:35, 57:37-57:44, 57:46-57:50, 57:52-57:61, 57:63-57:68, 57:70-57:88, 57:90-57:93, 58:36
2⁵	220 bp	800 bp	*57:01:01:01-57:01:24	*57:03:01:01-57:03:03, 57:06-57:08, 57:10, 57:14:01-57:18, 57:20-57:23, 57:25-57:27, 57:29, 57:31-57:41, 57:43-57:51, 57:53-57:60, 57:62, 57:64-57:81, 57:84-57:93, 35:208, 35:317, 40:30, 40:34, 44:153, 55:14, 58:14
3⁴	95 bp 170 bp 215 bp	800 bp		*57:04:01-57:04:02, 57:32, 44:153 *57:06, 57:18 *57:27
4⁴	90 bp 105 bp 170 bp	1070 bp		*57:20, 57:62 *57:15, 57:29, 07:120, 15:214, 18:81, 35:250, 40:150, 51:165 *57:91
5⁴	90 bp 165 bp 200 bp 245 bp	800 bp		*57:16, 57:34, 58:54 *57:23 *57:46, 57:64 *57:07, 57:26, 57:93, 08:181, 44:153, 55:14, C*06:72
6⁴	90 bp	1070 bp		*57:02:01-57:03:03, 57:07, 57:09, 57:12, 57:17, 57:39, 57:42, 57:46, 57:57, 57:63, 57:66, 57:70, 57:80, 57:84, 08:181, 40:30, 40:34 *57:08, 57:65, 15:403
7⁴	205 bp 100 bp 140 bp 185 bp 215 bp 250 bp	1070 bp		*57:53 *57:13, 57:31, 07:227, 08:181, 40:30, 40:34, C*06:72 *57:50 *57:09, 57:24, 08:181 *57:13, 57:14:01-57:14:02, 57:25, 07:227, 40:30, 40:34
8	160 bp 210 bp	1070 bp		*57:87 *57:10, 57:39, 57:51, 57:73, 07:219, 58:41
9⁴	110 bp 150 bp	800 bp		*57:33 *57:21, 57:40, 57:74, 14:20, 35:127, 50:54, 51:186, 53:41

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10	205 bp	1070 bp	*57:13, 57:22, 57:57, 57:63, 07:227, 55:14, C*06:72^w *57:43
	240 bp		
11⁴	100 bp	1070 bp	*57:01:01:01- 57:01:24 *57:06, 57:08, 57:10, 57:13-57:16, 57:18-57:27, 57:29-57:31, 57:33- 57:38, 57:40 ^w , 57:41, 57:43-57:45, 57:47-57:52, 57:54-57:56, 57:58- 57:62, 57:64-57:65, 57:67:01- 57:69, 57:71-57:75, 57:77-57:79N, 57:81, 57:85-57:93, 35:208, 35:317, 55:14, 58:14, C*06:72
12^{4,6}	75 bp	1070 bp	*57:35
	140 bp		*57:08, 57:60, 15:87, 15:403
13⁴	105 bp	1070 bp	*57:45, 57:51, 57:69, 57:71, 57:88, A*02:285
	135 bp		*57:58, 57:80, 57:90, 07:219, 58:12, A*02:42, A*02:310, A*02:597
	200 bp		*57:49
14⁴	115 bp	1070 bp	*57:54, 15:33, 15:248, 49:22, 51:126, C*03:87:01-03:87:02, C*05:27, C*05:39, C*08:115
	135 bp		*57:47
	205 bp		*57:77, 44:36, C*03:232
15	145 bp	1070 bp	*57:56, 57:78, 15:340, 35:300, 40:218, 45:21, 46:28, 51:58, 58:83
	185 bp		*57:48, A*23:72, A*33:12
	295 bp		*57:55
16⁴	65 bp	1070 bp	*57:67:01, 58:14, C*06:72
17⁴	90 bp	1070 bp	*57:04:01-57:04:02, 57:41, 44:153, C*06:72
	180 bp		*57:68
18⁶	170 bp	1070 bp	*57:37, 57:79N
	275 bp		*57:81, 44:13, 44:67, 51:170
	300 bp		*57:86, 53:25
19⁴	100 bp	1070 bp	*57:76
	165 bp		*57:92
	215 bp		*57:72
20	240 bp	1070 bp	*57:44, 44:249
	340 bp		*57:75, 40:327[?]
21⁴	100 bp	1070 bp	*57:36
	135 bp		*57:38
22⁶	210 bp	1070 bp	*57:85
23	180 bp	1070 bp	*57:59, 40:30, 40:34
24⁷	-	-	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-B*57:01 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.

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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 mix 2 may give rise to a lower yield of HLA-specific PCR product than the other B*57:01 primer mixes.

⁶Primer mixes 12, 18 and 22 have tendencies of unspecific amplifications.

⁷Primer mix 24 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 of the primer matching region not known.

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	90	220	95	90	90	90	100	160	110	205	100	75
			170	105	165	205	140	210	150	240		140
			215	170	200		185					
					245		215					
							250					
Length of int. pos. control ¹	800	800	800	1070	800	1070	1070	1070	800	1070	1070	1070
5'-primer(s) ²	209	362	362	209	130	320	362	97	349	362	362	209
	5'-ggC 3'	5'-ggT 3'	5'-ggT 3'	5'-ggC 3'	5'-AgT 3'	5'-CCC 3'	5'-ggT 3'	5'-TCC 3'	5'-CTT 3'	5'-ggT 3'	5'-ggT 3'	5'-ggC 3'
				646	200	362		103	352			
				5'-ACA 3'	5'-TCg 3'	5'-ggT 3'		5'-CCT 3'	5'-ACg 3'			
				704	209			148	353			
				5'-TgT 3'	5'-ggA 3'			5'-TgA 3'	5'-CAA 3'			
					362				392			
					5'-ggT 3'				5'-CgA 3'			
3'-primer(s) ³	256	539	418	259	256	2 nd I	421	266	463	527	419	244
	5'-CCC 3'	5'-TCA 3'	5'-gTC 3'	5'-CTT 3'	5'-CCC 3'	5'-TCg 3'	5'-ggT 3'	5'-TCC 3'	5'-gCT 3'	5'-CCT 3'	5'-Cgg 3'	5'-CTT 3'
			481	263	523	412	463			559	419	302
			5'-gTA 3'	5'-gTT 3'	5'-ACA 3'	5'-gTT 3'	5'-gCg 3'			5'-Cgg 3'	5'-CAG 3'	5'-ggC 3'
			500	271	559	521	505					320
			5'-ggA 3'	5'-CAC 3'	5'-CgT 3'	5'-ggA 3'	5'-gCC 3'					5'-Agg 3'
			537	774	572		538					
			5'-Agg 3'	5'-ggT 3'	5'-gCg 3'		5'-gTC 3'					
							559					
							5'-CTC 3'					
							583					
							5'-gTg 3'					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15	16	17	18	19	20	21	22	23
Length of spec. PCR product	105	115	145	65	90	170	100	240	100	210	180
	135	135	185		180	275	165	340	135		
	200	205	295			300	215				
Length of int. pos. control ¹	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070
5'-primer(s) ²	97	148	106	362	362	230	362	49	209	390	362
	5'-TCg 3'	5'-TgA 3'	5'-CCA 3'	5'-ggT 3'	5'-ggT 3'	5'-Agg 3'	5'-ggT 3'	5'-CAG 3'	5'-ggC 3'	5'-gAg 3'	5'-ggT 3'
	142	395	395			255					
	5'-TCT 3'	5'-gCT 3'	5'-gCC 3'			5'-gAg 3'					
	160	467	757			877					
	5'-ACT 3'	5'-CTg 3'	5'-CCA 3'			5'-AC 3'					
	193	485				878					
	5'-CCA 3'	5'-CAA 3'				5'-gCA 3'					
3'-primer(s) ³	256	559	212	387	409	2 nd I	419	122	268	559	499
	5'-CCC 3'	5'-CAG 3'	5'-ggT 3'	5'-TCg 3'	5'-ATA 3'	5'-TCg 3'	5'-CgA 3'	5'-CgA 3'	5'-gTg 3'	5'-CAG 3'	5'-ggA 3'
			214		502	916	488	220	302		
			5'-CCA 3'		5'-CTT 3'	5'-gAC 3'	5'-CCT 3'	5'-TgT 3'	5'-ggg 3'		
			539				534				
			5'-TCA 3'				5'-Tgg 3'				
			916								
			5'-gAC 3'								
Well No.	13	14	15	16	17	18	19	20	21	22	23

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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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[illegible]

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CELL LINE VALIDATION SHEET												
HLA-B*57:01 SSP typing kit ²												
					Prod. No.:	Well						
						17	18	19	20	21	22	23
						201893717	201893718	201893719	201893720	201893721	201893722	201893723
	IHWC cell line ¹			B*								
1	9001	SA	*07:02			-	-	-	-	-	-	-
2	9280	LK707	*52:01	*73:01		-	-	-	-	-	-	-
3	9011	E4181324	*52:01			-	-	-	-	-	-	-
4	9275	GU373	*15:10	*53:01		-	-	-	-	-	-	-
5	9009	KAS011	*37:01			-	-	-	-	-	-	-
6	9353	SM	*39:01	*51:01		-	-	-	-	-	-	-
7	9020	QBL	*18:01			-	-	-	-	-	-	-
8	9025	DEU	*35:01			-	-	-	-	-	-	-
9	9026	YAR	*38:01			-	-	-	-	-	-	-
10	9107	LKT3	*54:01			-	-	-	-	-	-	-
11	9051	PITOUT	*44:03			-	-	-	-	-	-	-
12	9052	DBB	*57:01			-	-	-	-	-	-	-
13	9025	JESTHOM	*27:05			-	-	-	-	-	-	-
14	9071	OLGA	*15:01	*15:20		-	-	-	-	-	-	-
15	9075	DKB	*40:01			-	-	-	-	-	-	-
16	9037	SWEIG007	*40:02			-	-	-	-	-	-	-
17	9282	CTM3953540	*08:01	*55:01		-	-	-	-	-	-	-
18	9257	32367	*14:01	*56:01		-	-	-	-	-	-	-
19	9038	BM16	*18:01			-	-	-	-	-	-	-
20	9059	SLE005	*40:01			-	-	-	-	-	-	-
21	9064	AMALA	*15:01			-	-	-	-	-	-	-
22	9056	KOSE	*35:03			-	-	-	-	-	-	-
23	9124	IHL	*40:02	*56:02		-	-	-	-	-	-	-
24	9035	JBUSH	*38:01			-	-	-	-	-	-	-
25	9049	IBW9	*14:02			-	-	-	-	-	-	-
26	9285	WT49	*58:01			-	-	-	-	-	-	-
27	9191	CH1007	*07:05	*51:01		-	-	-	-	-	-	-
28	9320	BEL5GB	*44:02	*44:03		-	-	-	-	-	-	-
29	9050	MOU	*44:03			-	-	-	-	-	-	-
30	9021	RSH	*42:01			-	-	-	-	-	-	-
31	9019	DUCAF	*18:01			-	-	-	-	-	-	-
32	9297	HAG	*41:02			-	-	-	-	-	-	-
33	9098	MT14B	*40:01			-	-	-	-	-	-	-
34	9104	DHIF	*38:01			-	-	-	-	-	-	-
35	9302	SSTO	*44:02			-	-	-	-	-	-	-
36	9024	KT17	*15:01	*35:01		-	-	-	-	-	-	-
37	9065	HHKB	*07:02			-	-	-	-	-	-	-
38	9099	LZL	*15:01			-	-	-	-	-	-	-
39	9315	CML	*08:01	*27:05		-	-	-	-	-	-	-
40	9134	WHONP199	*13:02	*46:01		-	-	-	-	-	-	-
41	9055	H0301	*14:02			-	-	-	-	-	-	-
42	9066	TAB089	*46:01			-	-	-	-	-	-	-
43	9076	T7526	*46:01			-	-	-	-	-	-	-
44	9057	TEM	*38:01			-	-	-	-	-	-	-
45	9239	SHJO	*42:01	*50:01		-	-	-	-	-	-	-
46	9013	SCHU	*07:02			-	-	-	-	-	-	-
47	9045	TUBO	*51:01			-	-	-	-	-	-	-
48	9303	TER-ND	*35:01	*44:03		-	-	-	-	-	-	-

101.572-12 – including *Taq* pol., IFU-01
101.572-12u – without *Taq* pol., IFU-02

Visit <https://labproducts.caredx.com> for
“Instructions for Use” (IFU)

Lot No.: 5G6

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 3 to 5, 7 to 10 and 12 to 23 were available. The specificities of the primers in primer solutions 3 to 5, 7, 8, 10, 12 to 17, 19 and 23 were tested by separately adding additional 5'-primers, respectively 3'-primers. In primer solutions 9, 18 and 22 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 20 and 21 it was only possible to test the 5'-primer, the 3'-primers were not possible to test. In primer solutions 3 to 7, 10 to 12, 15 and 19 one to three 3'-primers were not possible to test, and in primer solutions 4, 5, 8 and 13 to 15 one to three 5'-primers were not possible to test.

101.572-12 – including *Taq* pol., IFU-01
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Lot No.: **5G6**

Lot-specific information

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