

101.331-24/06 – including *Taq* pol., IFU-01
101.331-24u/06u – without *Taq* pol., IFU-02

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

Lot No.: **5G2**

Lot-specific information
Olerup SSP[®] DPA1

Product number:	101.331-24/06 – including <i>Taq</i> pol. 101.331-24u/06u – without <i>Taq</i> pol.
Lot number:	5G2
Expiry date:	2020-12-01
Number of tests:	24 tests – Product No. 101.331-24/24u 6 tests – Product No. 101.331-06/06u
Number of wells per test:	19+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. 5G2.

Complete product documentation consists of generic Instructions for Use (IFU), lot specific Product Insert, Worksheet and Certificate.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP[®] DPA1 LOT (2E0)

Four wells have been added to DPA1, wells **17 to 20**.

The format of the Worksheet has been changed.

The DPA1 specificity and interpretation tables have been updated for the DPA1 alleles described since the previous *Olerup SSP[®] DPA1* lot was made (**Lot No. 2E0**). The kit design is based on IMGT/HLA database 3.32.0.

101.331-24/06 – including *Taq* pol., IFU-01
101.331-24u/06u – without *Taq* pol., IFU-02

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Lot No.: **5G2**

Lot-specific information

The primers of the wells detailed below have been added, exchanged or modified.

Well	5'-primer	3'-primer	rationale
13	Modified	Modified	Modified primer pair added for improved resolution of the DPA1*02:06 allele.
16	Added	Added	Negative Control moved to well 20. Primer pair added for the DPA1*02:07:01-02:07:03 allele.
17	New	New	New primer pair for the DPA1*04:02 allele.
18	New	New	New primer pair for the DPA1*02:09 allele.
19	New	New	New primer pair for the DPA1*01:14 allele and the DPA1*02:08 allele.
20	-	-	Negative Control added from well 16.

101.331-24/06 – including *Taq* pol., IFU-01
101.331-24u/06u – without *Taq* pol., IFU-02

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Lot No.: **5G2**

Lot-specific information

Well **20** 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

DPA1 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DPA1*01:03 to DPA1*04:02 alleles.

PLATE LAYOUT

Each test consists of 20 PCR reactions in a 24 well cut PCR plate. Wells 21 to 24 are empty.

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

The 24 well cut PCR plate is marked with 'DPA1' in silver/gray ink.

Well No. 1 is marked with the Lot No. '5G2'.

Wells 1 to 19 – DPA1 high resolution primers.

Well 20 – 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

Only DPA1 alleles will be amplified by the DPA1 typing kit. Thus, the interpretation of DPA1 typings is not influenced by the DPA2 gene.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the phenotypically different DPA1 alleles, i.e. **DPA1*01:03 to DPA1*01:14**, **DPA1*02:01 to DPA1*02:09**, **DPA1*03:01 to DPA1*03:03** and **DPA1*04:01 to DPA1*04:02**, recognized by the HLA Nomenclature Committee in April 2018^{1,2} will give rise to unique amplification patterns by the primers in the DPA1 typing kit.

¹DPA1 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>.

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Lot No.: **5G2**

Lot-specific information

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DPA1 homo- and heterozygotes is available upon request.

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101.331-24u/06u – without *Taq* pol., IFU-02

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Lot No.: **5G2**

Lot-specific information
SPECIFICITY TABLE

DPA1 SSP typing

Specificities and sizes of the PCR products of the 19+1 primer mixes used for DPA1 SSP typing

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DPA1 ³ alleles
1⁴	85 bp	515 bp	*01:03:01:01-01:03:02, 01:03:04-01:05, 01:07-01:14, 04:01-04:02
2	255 bp	515 bp	*01:03:01:01-01:04, 01:06:01-01:12, 01:14
3	205 bp	430 bp	*01:03:01:01-01:03:05, 01:06:01-01:07, 01:09-01:14, 03:01:01-03:02
4^{4,5}	115 bp	430 bp	*01:04, 01:08, 03:03
5^{4,8}	105 bp	430 bp	*01:05, 02:01:01:01-02:09, 04:01-04:02
6	160 bp 195 bp 255 bp	515 bp	*01:10, 02:04 *01:06:01-01:06:02 *01:13
7⁴	100 bp	430 bp	*01:06:01-01:06:02, 02:01:01:01-02:01:08:02, 02:08-02:09
8⁴	100 bp	430 bp	*02:02:02:01-02:02:06, 02:04-02:07:01:03
9	205 bp	430 bp	*02:02:02:01-02:02:06, 02:04-02:07:01:03, 03:02
10^{4,7,8}	85 bp	515 bp	*01:03:03, 02:03, 03:01:01-03:03
11^{4,6}	90 bp 135 bp	515 bp	*01:12, 03:01:01-03:01:02, 03:03 *01:07
12	205 bp	430 bp	*04:01-04:02
13^{4,6}	90 bp	430 bp	*01:09, 02:06
14	130 bp	515 bp	*01:07-01:08, 01:11, 02:05
15	245 bp	430 bp	*01:03:03, 03:01:01-03:03
16	150 bp	430 bp	*02:01:03 [?] -02:01:07 [?] , 02:02:03 [?] -02:05 [?] , 02:07:01:01-02:07:01:03
17⁴	85 bp	430 bp	*02:01:01:01-02:01:02:02, 02:01:03 [?] -02:01:07 [?] , 02:01:08:01-02:02:02:04, 02:02:03 [?] -02:05 [?] , 02:06-02:09, 03:01:01, 03:02 [?] -03:03 [?] , 04:02
18⁴	120 bp	430 bp	*02:09
19	135 bp	430 bp	*01:14, 02:08
20⁹	-	-	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 DPA1 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

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101.331-24u/06u – without *Taq* pol., IFU-02

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“Instructions for Use” (IFU)

Lot No.: 5G2

Lot-specific information

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 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the longer, 515 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 DPA1 alleles 1st and/or 3rd 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.

⁴Specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁵Primer mix 4 may faintly amplify the DPA1*04:01 allele.

⁶Primer mixes 11 and 13 may have tendencies of unspecific amplifications.

⁷Primer mix 10 may give rise to a lower yield of HLA-specific PCR product than the other DPA1 primer mixes.

⁸Primer mixes 5 and 10 have tendencies to giving rise to primer oligomer formations.

⁹Primer mix 20 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.

‘?’, nucleotide sequence of the primer matching sequence is not known.

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Lot No.: **5G2**

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	85	255	205	115	105	160	100	100	205	85	90	205
PCR product						195					135	
						255						
Length of int.	515	515	430	430	430	515	430	430	430	515	515	430
pos. control ¹												
5'-primer(s) ²	15(138) 5'-ACg 3'	11(125) 5'-CgC 3'	28(177) 5'-gAA 3'	4(103) 5'-Cgg 3'	84(345) 5'-AAT 3'	11(125) 5'-CgT 3'	11(125) 5'-CgC 3'	11(125) 5'-CAT 3'	11(125) 5'-CAT 3'	15(138) 5'-ACC 3'	51(244) 5'-AAA 3'	18(145) 5'-gAA 3'
						31(185) 5'-gCA 3'					66(290) 5'-ATC 3'	
						43(222) 5'-TgT 3'						
3'-primer(s) ³	31(184) 5'-CAT 3'	83(340) 5'-ggT 3'	83(340) 5'-ggT 3'	28(177) 5'-TCg 3'	2 nd I 5'-ggC 3'	69(298) 5'-gTC 3'	31(184) 5'-CTg 3'	31(184) 5'-CTg 3'	66(290) 5'-TCA 3'	31(184) 5'-CAT 3'	83(340) 5'-ggT 3'	73(310) 5'-AgC 3'
						83(340) 5'-ggT 3'						
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15	16	17	18	19
Length of spec.	90	130	245	150	85	120	135
PCR product							
Length of int.	430	515	430	430	430	430	430
pos. control ¹							
5'-primer(s) ²	4(103) 5'-Cgg 3'	50(242) 5'-CCg 3'	15(138) 5'-ACC 3'	91(364) 5'-CCA 3'	190(661) 5'-CAA 3'	50(242) 5'-CCg 3'	190(661) 5'-CAA 3'
	190(662) 5'-AAT 3'	51(244) 5'-AAA 3'					
3'-primer(s) ³	23(161) 5'-ACg 3'	76(320) 5'-AAT 3'	83(340) 5'-ggT 3'	127(473) 5'-CCg 3'	204(705) 5'-CCC 3'	76(319) 5'-ACA 3'	218(746) 5'-AAT 3'
	204(705) 5'-CCC 3'	83(340) 5'-ggT 3'					224(764) 5'-CCT 3'
Well No.	13	14	15	16	17	18	19

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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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Lot No.: **5G2**

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CELL LINE VALIDATION SHEET																				
DPA1 SSP kit ²																				
					Well															
				Prod No.:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
					201670601	201670602	201670603	201670604	201670605	201670606	201670607	201670608	201670609	201670610	201670611	201670612	201893313	201670614	201670615	201893316
	IHWC cell line ¹		DPA1																	
1	9001	SA	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*02:01	*04:01	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-
5	9009	KAS011	*01:03	*02:01	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-
6	9353	SM	*02:02		-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-
7	9020	QBL	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025	DEU	*01		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*02:02		-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-
11	9051	PITOUT	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*01:03	*02:01	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-
18	9257	32367	*01:03	*03:01	+	+	+	-	-	-	-	-	-	+	+	-	-	-	+	-
19	9038	BM16	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*01:03	*02:01	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-
23	9124	IHL	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*02:01		-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
26	9285	WT49	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*01:03	*04:01	+	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-
28	9320	BEL5GB	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*02:02	*03:01	-	-	+	-	+	-	-	+	+	+	+	-	-	-	+	-
31	9019	DUCAF	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*02:02		-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-
37	9065	HHKB	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*01:03	*02:01	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*02:02		-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-
41	9055	H0301	*02:01		-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*02:02		-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-
43	9076	T7526	*04:01		+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
44	9057	TEM	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*01:03	*03:01	+	+	+	-	-	-	-	-	-	+	+	-	-	-	+	-
46	9013	SCHU	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*01:03		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-

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Lot-specific information

CELL LINE VALIDATION SHEET							
DPA1 SSP kit ²							
					Well		
					17	18	19
				Prod No.:	201893317	201893318	201893319
	IHWC cell line ¹		DPA1				
1	9001	SA	*01:03		-	-	-
2	9280	LK707	*01:03		-	-	-
3	9011	E4181324	*01:03		-	-	-
4	9275	GU373	*02:01	*04:01	+	-	-
5	9009	KAS011	*01:03	*02:01	+	-	-
6	9353	SM	*02:02		+	-	-
7	9020	QBL	*01:03		-	-	-
8	9025	DEU	*01		-	-	-
9	9026	YAR	*01:03		-	-	-
10	9107	LKT3	*02:02		+	-	-
11	9051	PITOUT	*01:03		-	-	-
12	9052	DBB	*01:03		-	-	-
13	9004	JESTHOM	*01:03		-	-	-
14	9071	OLGA	*01:03		-	-	-
15	9075	DKB	*01:03		-	-	-
16	9037	SWEIG007	*01:03		-	-	-
17	9282	CTM3953540	*01:03	*02:01	+	-	-
18	9257	32367	*01:03	*03:01	+	-	-
19	9038	BM16	*01:03		-	-	-
20	9059	SLE005	*01:03		-	-	-
21	9064	AMALA	*01:03		-	-	-
22	9056	KOSE	*01:03	*02:01	+	-	-
23	9124	IHL	*01:03		-	-	-
24	9035	JBUSH	*01:03		-	-	-
25	9049	IBW9	*02:01		+	-	-
26	9285	WT49	*01:03		-	-	-
27	9191	CH1007	*01:03	*04:01	-	-	-
28	9320	BEL5GB	*01:03		-	-	-
29	9050	MOU	*01:03		-	-	-
30	9021	RSH	*02:02	*03:01	+	-	-
31	9019	DUCAF	*01:03		-	-	-
32	9297	HAG	*01:03		-	-	-
33	9098	MT14B	*01:03		-	-	-
34	9104	DHIF	*01:03		-	-	-
35	9302	SSTO	*01:03		-	-	-
36	9024	KT17	*02:02		+	-	-
37	9065	HHKB	*01:03		-	-	-
38	9099	LZL	*01:03		-	-	-
39	9315	CML	*01:03	*02:01	+	-	-
40	9134	WHONP199	*02:02		+	-	-
41	9055	H0301	*02:01		+	-	-
42	9066	TAB089	*02:02		+	-	-
43	9076	T7526	*04:01		-	-	-
44	9057	TEM	*01:03		-	-	-
45	9239	SHJO	*01:03	*03:01	+	-	-
46	9013	SCHU	*01:03		-	-	-
47	9045	TUBO	*01:03		-	-	-
48	9303	TER-ND	*01:03		-	-	-

101.331-24/06 – including *Taq* pol., IFU-01
101.331-24u/06u – without *Taq* pol., IFU-02

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Lot No.: **5G2**

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 allele to be amplified by primer solutions 6,13,14,18 and 19 were available. The specificities of the primers in primer solutions 6, 13, 14, were tested by separately adding one additional 5'-primer and one additional 3'-primer, respectively. In primer solutions 18 and 19 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 6, 11, 13 and 14 one or two 5'-primers were not possible to test, and in primer solutions 6, 13 and 14 one or two 3'-primers were not possible to test.

101.331-24/06 – including *Taq* pol., IFU-01
101.331-24u/06u – without *Taq* pol., IFU-02

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Lot No.: **5G2**

Lot-specific information

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