

101.901-24 – including *Taq* polymerase, IFU-01
 101.901-24u – without *Taq* polymerase, IFU-02

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

Lot No.: **1E7**

Lot-specific information

Olerup SSP® DQB1*06:02,DQA1*01:02 - SSP

Product number:	101.901-24 – including <i>Taq</i> polymerase 101.901-24u – without <i>Taq</i> polymerase
Lot number:	1E7
Expiry date:	2019-02-01
Number of tests:	24
Number of wells per test:	7+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. 1E7.

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

**CHANGES COMPARED TO THE PREVIOUS OLERUP SSP®
 DQB1*06:02,DQA1*01:02 LOT (47X)**

The DQB1*06:02,DQA1*01:02 kit has been redesigned and improved with regards to allelic detection and discrimination and facilitated interpretation. The kit resolution focuses on common and well documented (CWD) alleles¹.

The new lot contains 7 primer mixes for the DQA1, DQB1 and DRB1 alleles and includes a negative control.

Positive and negative control DNAs are included in the kit.

The DQB1*06:02,DQA1*01:02 specificity and interpretation tables have been updated for the DQA1, DQB1 and DRB1 alleles described since the previous *Olerup SSP®* DQB1*06:02,DQA1*01:02 lot (**Lot No. 47X**) was made. The kit design is based on IMGT/HLA database 3.24.0.

¹S. J. Mack¹, P. Cano², J. A. Hollenbach¹ et al.
 Common and well-documented HLA alleles: 2012 update to the CWD catalogue. *Tissue Antigens*, 2013, 81, 194–203



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

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

Well	5'-primer	3'-primer	rationale
7	Exchanged	Exchanged	Primer pairs exchanged for resolution of the DRB1*15:127 and 15:129N alleles.

Changes in revision R01 compared to R00:

1. Primer mix 7 does not amplify the DRB1*16:05:01-16:05:02 and 16:07 alleles. This has been corrected in the Specificity and Interpretation Tables.

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Well **8** 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

DQB1*06:02,DQA1*01:02 - SSP

CONTENT

The primer set contains 5'- and 3'-primers for identifying the following on CWD level^{1,2}:

DQB1*06:02 DQA1*01:02 DRB1*15:xx

DQB1*06:02 DQA1*01:02

DQB1*06:02 DRB1*15:xx

DQB1*06:02

DQA1*01:02

DRB1*15:xx

¹Mahlis J et al.

The autoimmune basis of narcolepsy.

Current Opinion in Neurobiology 2013, 23:767–773

²Mignot E et al.

HLA DQB1*0602 is associated with cataplexy in 509 narcoleptic patients.

Sleep 1997, 20:1012-1020.

Please note that DQB1 amplifications usually are somewhat less pronounced than e.g. DRB and DQA1 amplifications even when using the same DNA preparation and exactly the same experimental procedures.

Positive and negative control DNAs are included in the kit:

DNA 1; positive control DNA **IHW 9013, SCHU**. (The positive control DNA will be positive for the alleles DQA1*01:02, DQB1*06:02)

DNA 2; negative control DNA, **IHW 9065, HHKB**, DQA1*01:03, DQB1*06:03.

(A DQA1*01:03, DQB1*06:03-positive DNA was chosen as negative control, as this is most similar to the DQA1*01:02, DQB1*06:02 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 8 PCR reactions in a 8 well PCR plate

1	2	3	4	5	6	7	8
DQA1	DQA1	DQA1	DQA1	DQB1	DQB1	DRB1	NC

The 8 well PCR plate is marked with '1E7' in silver/gray ink.

Well No. 1 is marked with the Lot No. '1E7'.

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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.

Wells 1 to 4 – DQA1 high resolution primers.

Wells 5 to 6 – DQB1 high resolution primers.

Well 7 – DRB1 low resolution primers.

Well 8 – Negative Control (NC).

The PCR plates are covered with a PCR-compatible foil.

Please note: When removing each 8 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.

UNIQUELY IDENTIFIED ALLELES

DQA1, DQB1 and DRB1 alleles recognized by the HLA Nomenclature Committee in April 2016^{1,2} have been considered in the specificity and interpretation tables of the DQB1*06:02,DQA1*01:02 kit.

For further details see Content section.

The DQB1*06:02,DQA1*01:02 kit cannot distinguish the DQB1*06:02:01-06:02:04 and 06:02-06-06:02:25 alleles or the DQA1*01:02:01-01-01:02:04 alleles.

¹DQA1, DQB1 and DRB1 alleles listed on the IMGT/HLA web page 2016-April-15, release 3.24.0, 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>.

EXPECTED RESULTS

Table 1 describes expected results for the (groups of) alleles that the kit is able to detect and separate.

Table 1: Expected results for targeted DQA1, DQB1 and DRB1 alleles.

DQA1 alleles	DQB1 alleles	DRB1 alleles	Positive DQA1 wells	Positive DQB1 wells ¹	Positive DRB1 wells
*01:02	*06:02	*15:xx	2, 3	5	7
	*06:02	*15:xx		5	7
*01:02	*06:02		2, 3	5	
*01:02		*15:xx	2, 3		7
	*06:02			5	
*01:02			2, 3		
		*15:xx			7

¹The DQB1*06:02:05 allele is also amplified in well 6.

In the negative control well no PCR product should be seen. The presence of PCR product(s) indicates contamination. Primer oligomer artifacts, approximately 40 to 50 bp in size, may be seen. This does not represent contamination.

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SPECIFICITY TABLE

DQB1*06:02,DQA1*01:02 - SSP

Specificities and sizes of the PCR products of the 7+1 primer mixes used for DQB1*06:02,DQA1*01:02 SSP typing

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA class II alleles ³
1⁴	145 bp	515 bp	DQA1*01:01:01:01:01:01:03, DQA1*01:04:01:01:01:05:02, DQA1*01:07Q, DQA1*01:12
2	170 bp	515 bp	DQA1*01:01:01:01:01:02:04, DQA1*01:04:01:01:01:09, DQA1*01:11-01:13
3⁴	145 bp	430 bp	DQA1*01:02:01:01:01:03:01:06, DQA1*01:06, DQA1*01:08-DQA1*01:11, DQA1*01:13
4⁴	170 bp	430 bp	DQA1*01:03:01:01:01:03:01:06, DQA1*01:10
5	185 bp	430 bp	DQB1*04:10, DQB1*06:02:01-06:02:25, DQB1*06:14:01-DQB1*06:16, DQB1*06:19:01-06:20, DQB1*06:23-06:24, DQB1*06:33, DQB1*06:37, DQB1*06:46-06:50, DQB1*06:51:02, DQB1*06:68, DQB1*06:70-06:84, DQB1*06:95, DQB1*06:97, DQB1*06:107, DQB1*06:109, DQB1*06:111-06:117, DQB1*06:122, DQB1*06:124-06:127, DQB1*06:136-06:138, DQB1*06:146:01-06:147, DQB1*06:150-06:152, DQB1*06:156, DQB1*06:159, DQB1*06:161-06:163, DQB1*06:166, DQB1*06:173-06:175, DQB1*06:178-06:179N, DQB1*06:182-06:183, DQB1*06:188, DQB1*06:192, DQB1*06:197-06:198, DQB1*06:200-06:201
6	155 bp	430 bp	DQB1*03:30, DQB1*03:72, DQB1*03:100, DQB1*03:132, DQB1*03:215, DQB1*04:01:01-04:02:04, DQB1*04:02:06, DQB1*04:02:08-04:03:02, DQB1*04:06-04:08, DQB1*04:10, DQB1*04:12-04:14, DQB1*04:16-04:34, DQB1*05:38, DQB1*05:119, DQB1*06:02:05, DQB1*06:19:01, DQB1*06:139
7	200 bp, 230 bp	430 bp	DRB1*15:01:01:01-15:131
8^{5,6}	-	-	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 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 inherit 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

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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 shorter, 430 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 DQA1, DQB1 and DRB1 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.

⁴Primer mixes 1, 3 and 4 may give rise to a lower yield of HLA-specific PCR product than the other DQB1*06:02,DQA1*01:02 primer mixes.

⁵Primer mix 8 has a tendency to giving rise to primer oligomer formation.

⁶Primer mix 8 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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DQA1 PRIMER SPECIFICATION

Well No.	1	2	3	4
Length of spec. PCR product	145	170	145	170
Length of int. pos. control ¹	515	515	430	430
5'-primer(s) ²	33(169) 5' -Agg 3'	25(143) 5' -gTA 3'	33(169) 5' -AgC 3'	25(143) 5' -gTT 3'
3'-primer(s) ³	68(274) 5' -TgC 3'	68(274) 5' -TgC 3'	68(274) 5' -TgC 3'	68(274) 5' -TgC 3'
Well No.	1	2	3	4

DQB1 PRIMER SPECIFICATION

Well No.	5	6
Length of spec. PCR product	185	155
Length of int. pos. control ¹	430	430
5'-primer(s) ²	9(122) 5' -gTT 3'	9(122) 5' -gTT 3'
3'-primer(s) ³	57(266) 5' -CAT 3'	47(237) 5' -CgA 3'
	58(270) 5' -TCC 3'	
Well No.	5	6

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DRB1 PRIMER SPECIFICATION

Well No.	7
Length of spec.	200
PCR product	230
Length of int. pos. control ¹	430
5'-primer(s) ²	11(121)
	5'-CTg 3'
	13(126)
	5'-AgA 3'
	13(126)
	5'-AAg 3'
	13(126)
	5'-Agg 3'
3'-primer(s) ³	66(286)
	5'-gAT 3'
	66(286)
	5'-gAT 3'
	69(295)
	5'-.Tg 3'
	69(295)
	5'-CTg 3'
	70(298)
	5'-CgC 3'
	71(299)
	5'-gCT 3'
	71(301)
	5'-.Cg 3'
	73(305)
	5'-Cgg 3'
	73(305)
	5'-ggC 3'
	77(317)
	5'-AgT 3'
Well No.	7

¹The 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 shorter, 430 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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CELL LINE VALIDATION SHEET								
DQA1*01:02								
				Prod. No.	Well			
					1	2	3	4
					201443101B	201443101	201443102	201443104
	IHWC cell line	DQA1						
1	9001 SA	*01:01			+	+	-	-
2	9280 LK707	*01:03	*03:03		-	-	+	+
3	9011 E4181324	*01:03			-	-	+	+
4	9275 GU373	*05:01			-	-	-	-
5	9009 KAS011	*01:02			-	+	+	-
6	9353 SM	*01:03	*03:01		-	-	+	+
7	9020 QBL	*05:01			-	-	-	-
8	9025 DEU	*03			-	-	-	-
9	9026 YAR	*03:01			-	-	-	-
10	9107 LKT3	*03:03			-	-	-	-
11	9051 PITOUT	*02:01			-	-	-	-
12	9052 DBB	*02:01			-	-	-	-
13	9004 JESTHOM	*01:01			+	+	-	-
14	9071 OLGA	*04:01			-	-	-	-
15	9075 DKB	*03:02			-	-	-	-
16	9037 SWEIG007	*05:05			-	-	-	-
17	9282 CTM3953540	*01:03	*05:01		-	-	+	+
18	9257 32367	*01:02	*03:03		-	+	+	-
19	9038 BM16	*05:05			-	-	-	-
20	9059 SLE005	*01:02			-	+	+	-
21	9064 AMALA	*05:03			-	-	-	-
22	9056 KOSE	*01:02	*01:04		+	+	+	-
23	9124 IHL	*01:03	*01:04		+	+	+	+
24	9035 JBUSH	*05:05			-	-	-	-
25	9049 IBW9	*02:01			-	-	-	-
26	9285 WT49	*05:01			-	-	-	-
27	9191 CH1007	*03:03	*01:05		-	+	-	-
28	9320 BEL5GB	*02:01	*03:03		-	-	-	-
29	9050 MOU	*02:01			-	-	-	-
30	9021 RSH	*04:01			-	-	-	-
31	9019 DUCAF	*05:01			-	-	-	-
32	9297 HAG	*05:05			-	-	-	-
33	9098 MT14B	*03:01			-	-	-	-
34	9104 DHIF	*05:05			-	-	-	-
35	9302 SSTO	*03:01			-	-	-	-
36	9024 KT17	*03:01			-	-	-	-
37	9065 HHKB	*01:03			-	-	+	+
38	9099 LZL	*05:03			-	-	-	-
39	9315 CML	*03:03	*05:01		-	-	-	-
40	9134 WHONP199	*02:01	*03:02		-	-	-	-
41	9055 H0301	*01:02			-	+	+	-
42	9066 TAB089	*01:03			-	-	+	+
43	9076 T7526	*03:02			-	-	-	-
44	9057 TEM	*01:04			+	+	-	-
45	9239 SHJO	*02:01	*03:03		-	-	-	-
46	9013 SCHU	*01:02			-	+	+	-
47	9045 TUBO	*05:05			-	-	-	-
48	9303 TER-ND	*01:01			+	+	-	-



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DQB1*06:02						
				Prod. No.	Well	
					5	6
					201443105	201443106
	IHC cell line		DQB1			
1	9001 SA		*05:01		-	-
2	9280 LK707		*06:01	*02:02	-	-
3	9011 E4181324		*06:01		-	-
4	9275 GU373		*02:01		-	-
5	9009 KAS011		*05:02		-	-
6	9353 SM		*03:02	*06:01	-	-
7	9020 QBL		*02:01		-	-
8	9025 DEU		*03:01		-	-
9	9026 YAR		*03:02		-	-
10	9107 LKT3		*04:01		-	+
11	9051 PITOUT		*02:02		-	-
12	9052 DBB		*03:03		-	-
13	9004 JESTHOM		*05:01		-	-
14	9071 OLGA		*04:02		-	+
15	9075 DKB		*03:03		-	-
16	9037 SWEIG007		*03:01		-	-
17	9282 CTM3953540		*02:01	*06:03	-	-
18	9257 32367		*06:02	*02:02	+	-
19	9038 BM16		*03:01		-	-
20	9059 SLE005		*06:04		-	-
21	9064 AMALA		*03:01		-	-
22	9056 KOSE		*05:03	*06:04	-	-
23	9124 IHL		*05:03	*06:01	-	-
24	9035 JBUSH		*03:01		-	-
25	9049 IBW9		*02:02		-	-
26	9285 WT49		*02:01		-	-
27	9191 CH1007		*04:01	*05:01	-	+
28	9320 BEL5GB		*02:02	*03:01	-	-
29	9050 MOU		*02:02		-	-
30	9021 RSH		*04:02		-	+
31	9019 DUCAF		*02:01		-	-
32	9297 HAG		*03:01		-	-
33	9098 MT14B		*03:02		-	-
34	9104 DHIF		*03:01		-	-
35	9302 SSTO		*03:05		-	-
36	9024 KT17		*03:02		-	-
37	9065 HHKB		*06:03		-	-
38	9099 LZL		*03:01		-	-
39	9315 CML		*02:01	*03:01	-	-
40	9134 WHONP199		*02:02	*03:03	-	-
41	9055 H0301		*06:09		-	-
42	9066 TAB089		*06:01		-	-
43	9076 T7526		*03:03		-	-
44	9057 TEM		*05:03		-	-
45	9239 SHJO		*02:02		-	-
46	9013 SCHU		*06:02		+	-
47	9045 TUBO		*03:01		-	-
48	9303 TER-ND		*05:01		-	-

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CELL LINE VALIDATION SHEET				
DRB1*15:01				
				Well
				7
				Prod. No.
				201670207
IHC cell line		DRB1		
1	9001 SA	*01:01		-
2	9280 LK707	*15:02	*04:05	+
3	9011 E4181324	*15:02		+
4	9275 GU373	*03:01		-
5	9009 KAS011	*16:01		-
6	9353 SM	*04:07	*08:03	-
7	9020 QBL	*03:01		-
8	9025 DEU	*04:01		-
9	9026 YAR	*04:02		-
10	9107 LKT3	*04:05		-
11	9051 PITOUT	*07:01		-
12	9052 DBB	*07:01		-
13	9004 JESTHOM	*01:01		-
14	9071 OLGA	*08:02		-
15	9075 DKB	*09:01		-
16	9037 SWEIG007	*11:01		-
17	9282 CTM3953540	*03:01	*13:01	-
18	9257 32367	*09:01	*11:01	-
19	9038 BM16	*12:01		-
20	9059 SLE005	*13:02		-
21	9064 AMALA	*14:02		-
22	9056 KOSE	*13:02	*14:54	-
23	9124 IHL	*08:03	*14:14	-
24	9035 JBUSH	*11:01		-
25	9049 IBW9	*07:01		-
26	9285 WT49	*03:01		-
27	9191 CH1007	*04:05	*10:01	-
28	9320 BEL5GB	*04:16	*07:01	-
29	9050 MOU	*07:01		-
30	9021 RSH	*03:02		-
31	9019 DUCAF	*03:01		-
32	9297 HAG	*13:03		-
33	9098 MT14B	*04:04		-
34	9104 DHIF	*11:01		-
35	9302 SSTO	*04:03		-
36	9024 KT17	*04:03	*04:06	-
37	9065 HHKB	*13:01		-
38	9099 LZL	*14:02		-
39	9315 CML	*03:01	*04:01	-
40	9134 WHONP199	*07:01	*09:01	-
41	9055 H0301	*13:02		-
42	9066 TAB089	*08:03		-
43	9076 T7526	*09:01		-
44	9057 TEM	*14:01		-
45	9239 SHJO	*07:01		-
46	9013 SCHU	*15:01		+
47	9045 TUBO	*11:04	*12:01	-
48	9303 TER-ND	*01:03		-

101.901-24 – including *Taq* polymerase, IFU-01

101.901-24u – without *Taq* polymerase, IFU-02

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Lot No.: 1E7

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.

In primer solutions 5 and 7 one or more 3'-primers were not possible to test, and in primer solution 7 three 5'-primers were not possible to test.

Additional 3'-primers in primer mix 7 were tested by separately adding one 5'-primer.

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Lot No.: **1E7**

Lot-specific information

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