

101.121-24/04 – including *Taq* pol., IFU-01
101.121-24u/04u – without *Taq* pol., IFU-02

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“Instructions for Use” (IFU)

Lot No.: **2E8**

Lot-specific information
Olerup SSP® DRB3

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Product number:	101.121-24/04 – including <i>Taq</i> pol. 101.121-24u/04u – without <i>Taq</i> pol.
Lot number:	2E8
Expiry date:	2019-03-01
Number of tests:	24 tests – Product No. 101.121-24/24u 4 tests – Product No. 101.121-04/04u
Number of wells per test:	38+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. 2E8.

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

**CHANGES COMPARED TO THE PREVIOUS OLERUP SSP®
DRB3 Lot (73Y)**

Seven wells have been added to DRB3, wells **33 to 39**

The DRB3 primer set as well as the specificity and interpretation tables have been updated for the DRB alleles described since the previous *Olerup SSP®* DRB3 lot (**Lot No. 73Y**) was made. The kit design is based on IMGT/HLA database 3.24.0.

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, added or modified compared to the previous lot.

Well	5'-primer	3'-primer	rationale
18	Added	-	5'-primer added for the DRB3*01:18 allele.
21	-	Added	3'-primer added for the DRB3*01:19 allele.
25	-	Added	3'-primer added for the DRB3*01:19 allele.
28	-	Added	3'-primer added for the DRB3*02:31 allele.
32	Added	Added	Primer pair added for the DRB3*03:04 allele, Negative Control moved to well 39.
33	New	New	New primer pairs for the DRB3*01:20 and DRB3*03:06 alleles.
34	New	New	New primer pairs for the DRB3*02:36 and DRB3*03:05 alleles.
35	New	New	New primer pairs for the DRB3*01:17 and DRB3*02:33 alleles.
36	New	New	New primer pairs for the DRB3*01:22 and DRB3*02:35 alleles.
37	New	New	New primer pair for the DRB3*02:30 allele.
38	New	New	New primer pairs for the DRB3*02:32 and DRB3*02:34 alleles.
39	-	-	Negative Control added from well 32.

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Well **39** 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

DRB3 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB3*01:01 to DRB3*03:06 alleles.

PLATE LAYOUT

Each test consists of 39 PCR reactions in a 48 well cut PCR plate. Wells 40 to 48 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32
33	34	35	36	37	38	NC	empty
empty	empty	empty	empty	empty	empty	empty	empty

The 48 well cut PCR plate is marked with 'DRB3' in silver/gray ink.

Well No. 1 is marked with the Lot No. '2E8'.

Wells 1 to 38 – DRB3 high resolution primers.

Well 39 – Negative Control (NC).

A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded. The PCR plates are covered with a PCR-compatible foil.

Please note: When removing each 48 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Due to the sharing of sequence motifs between DRB3 and DRB1 alleles, primer mixes 1 to 5, 7 to 17, 19, 20, 24, 26, 28, 29, 31 to 33 and 38 will amplify DRB1 alleles. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the DRB3 alleles, i.e. **DRB3*01:01 to DRB3*01:22, DRB3*02:01 to DRB3*02:36 and DRB3*03:01 to DRB3*03:06**, recognized by the HLA Nomenclature Committee in April 2016^{1,2} will give rise to unique amplification patterns by the primers in the DRB3 subtyping kit.

The DRB3 subtyping kit cannot distinguish the silent mutations in the DRB3*01:01:02:01-01:01:06 alleles, the DRB3*02:02:01:01-02:02:06 alleles or the DRB3*03:01:01-03:01:03 alleles.

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¹DRB 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>.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DRB3 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

DRB3 SSP subtyping

Specificities and sizes of the PCR products of the 38+1 primer mixes used for DRB3 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB3 alleles ³	Other amplified DRB alleles ⁴
1⁵	100 bp	515 bp	*01:01:02:01-01:08, 01:10-01:13, 01:15-01:21, 02:05, 03:01:01-03:01:03, 03:03-03:06	DRB1*03:42, DRB1*03:87, DRB1*11:30 ^w , DRB1*12:57, DRB1*13:67, DRB1*13:195, DRB1*14:46
2^{5,7}	125 bp	430 bp	*01:01:02:01-01:06, 01:08, 01:10-01:14, 01:16-01:22, 03:01:01-03:01:03, 03:03-03:06	DRB1*03:38, DRB1*03:79, DRB1*03:85, DRB1*03:96, DRB1*04:82 ^w , DRB1*04:99 ^w , DRB1*08:07 ^w , DRB1*08:19 ^w , DRB1*08:25 ^w , DRB1*08:34 ^w , DRB1*08:52 ^w , DRB1*12:27 ^w , DRB1*12:57, DRB1*13:31 ^w , DRB1*13:46 ^w , DRB1*13:54 ^w , DRB1*13:77 ^w , DRB1*13:100 ^w , 13:162 ^w , 13:180 ^w , DRB1*14:48 ^w , DRB1*14:174
3⁵	95 bp	430 bp	*01:01:02:01-01:02, 01:04-01:08, 01:10-01:13, 01:15-01:21	DRB1*03:42
4⁸	240 bp	430 bp		DRB1*11:13:01-11:13:02, DRB1*11:149, DRB1*13:08, DRB1*14:01:01, DRB1*14:01:02 [?] -14:01:04 [?] , DRB1*14:02:02 [?] , DRB1*14:04:01, DRB1*14:04:02 [?] -14:04:03 [?] , DRB1*14:05:01, DRB1*14:05:02 [?] -14:05:04 [?] , DRB1*14:06:02 [?] -14:06:03 [?] , DRB1*14:07:01, DRB1*14:07:02 [?] , DRB1*14:08, DRB1*14:09 [?] -14:10 [?] , DRB1*14:11, DRB1*14:12:01 [?] -14:12:02 [?] , DRB1*14:14, DRB1*14:15 [?] -14:20 [?] , DRB1*14:22 [?] -14:45 [?] , DRB1*14:47 [?] -14:53 [?] , DRB1*14:54:01-14:54:05, DRB1*14:55 [?] -14:56 [?] , 14:57, 14:58 [?] - DRB1*14:65 [?] , DRB1*14:67 [?] -14:81 [?] , DRB1*14:82, DRB1*14:83 [?] - DRB1*14:136 [?] , DRB1*14:137N, DRB1*14:138 [?] -14:140 [?] , DRB1*14:141, DRB1*14:142 [?] -14:143 [?] , DRB1*14:145 [?] -14:151 [?] , DRB1*14:152N, DRB1*14:153 [?] -14:170 [?] , DRB1*14:171, DRB1*14:172 [?] -14:174 [?]
5⁵	95 bp 125 bp	430 bp	*01:03 *01:10	DRB1*14:46
6	190 bp	430 bp	*01:04	
7^{5,7}	90 bp 180 bp	515 bp	*01:05 *01:07, 01:15	DRB1*03:42, DRB1*14:46
8⁵	120 bp	430 bp	*01:06, 01:08	DRB1*03:42, DRB1*14:46

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9^{6,7}	165 bp	430 bp	*01:07, 01:21, 02:01-02:08, 02:11- 02:36	DRB1*14:141
10^{6,8}	185 bp	515 bp	*01:09, 02:01- 02:04, 02:06-02:14, 02:16-02:26, 02:28- 02:36, 03:02	DRB1*03:101, DRB1*03:120, DRB1*12:22, DRB1*14:141
11⁷	270 bp	515 bp	*02:01, 02:04, 02:24, 03:01:01- 03:02, 03:04-03:05	DRB1*03:42, DRB1*03:87, DRB1*12:57
12⁵	90 bp 145 bp	515 bp	*02:17 *02:01, 02:04, 02:24	DRB1*03:37, DRB1*03:80, DRB1*04:183, DRB1*11:43, DRB1*11:50, DRB1*11:115, DRB1*11:151, DRB1*11:171, DRB1*11:174, DRB1*12:34, DRB1*13:131, DRB1*13:150, DRB1*14:59, DRB1*14:86, DRB1*14:96, DRB1*14:145, DRB1*14:153-14:154, DRB1*15:65
13⁶	270 bp	515 bp	*01:01:02:01-01:13, 01:15-01:22, 02:02:01:01-02:03, 02:05-02:13, 02:15- 02:23, 02:25-02:36, 03:03, 03:06	DRB1*11:30, DRB1*13:67, DRB1*13:195, DRB1*14:46, DRB1*14:141
14	185 bp	515 bp	*01:07, 01:09, 02:02:01:01- 02:02:06, 02:05- 02:13, 02:15-02:19, 02:21-02:23, 02:25- 02:36	DRB1*10:01:01:01^w-10:01:09^w, DRB1*10:03^w-10:11^w, DRB1*10:13^w, DRB1*10:15^w-10:16^w, DRB1*14:141, DRB1*16:30
15⁵	265 bp 120 bp	430 bp	*01:02 *02:03, 03:01:01- 03:06	DRB1*12:57, DRB1*13:195
16	180 bp 210 bp 150 bp	430 bp	*02:16 *02:17 *01:09, 02:04, 02:19, 02:22	DRB1*03:44
17⁵	120 bp	430 bp	*01:08, 02:06, 02:20	DRB1*03:42, DRB1*03:87, DRB1*13:67, DRB1*14:46
18⁵	180 bp 100 bp	430 bp	*02:16, 02:23 *01:09, 02:07, 02:09, 02:21	
19⁷	170 bp 180 bp	515 bp	*01:18 *02:08, 02:18, 02:23	DRB1*11:30
20⁷	270 bp 170 bp	430 bp	*02:14 *01:01:02:01-01:06, 01:08-01:13, 01:15- 01:20, 01:22, 02:09-02:10, 02:16, 03:01:01-03:06	DRB1*03:42, DRB1*03:87, DRB1*11:30, DRB1*12:57, DRB1*13:67, DRB1*13:195, DRB1*14:46
21⁵	120 bp 210 bp	430 bp	*01:13 *01:19, 02:11	
22⁷	185 bp 245 bp	430 bp	*01:11 *01:02, 02:12	

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Lot No.: 2E8		Lot-specific information		www.olerup-ssp.com
23	195 bp 240 bp	430 bp	*02:13 *01:02	
24	185 bp	430 bp	*02:09, 02:18- 02:19, 02:21, 02:25, 03:01:01- 03:06	DRB1*11:30, DRB1*12:57
25	225 bp 260 bp	430 bp	*01:19, 02:26 *01:12, 02:31	
26	235 bp	430 bp	*01:14	DRB1*03:01:02, DRB1*03:02:02, DRB1*03:05:03, DRB1*03:13:02, DRB1*03:17, DRB1*03:24, DRB1*03:27, DRB1*03:35, DRB1*03:81, DRB1*08:67, DRB1*11:27:01, DRB1*11:84:01, DRB1*11:124, DRB1*11:136, DRB1*11:138, DRB1*12:22, DRB1*13:33:02-13:33:03, DRB1*13:61:01, DRB1*13:94:02, DRB1*13:96:02, DRB1*14:38:01, DRB1*14:47, DRB1*14:50, DRB1*14:98, DRB1*14:127:01, DRB1*14:165
27⁵	90 bp	430 bp	*02:01	
28	235 bp	430 bp	*02:27	DRB1*11:30, DRB1*12:57, DRB1*13:67, DRB1*13:195, DRB1*14:46
29⁸	260 bp 160 bp	430 bp	*01:12, 02:31 *02:15, 02:27	DRB1*10:01:01:01^w-10:06^w, DRB1*10:08^w- 10:10^w, DRB1*10:12^w-10:13^w, DRB1*10:15^w- 10:16^w, DRB1*13:106, DRB1*16:30
30	210 bp	430 bp	*02:28	
31⁵	70 bp	430 bp	*01:21, 02:19, 02:21, 02:25	DRB1*12:34
32	95 bp 230 bp	430 bp	*01:16, 02:29N *02:04, 02:19, 02:22, 03:03-03:04	DRB1*03:87
33⁹	150 bp	430 bp	*03:01:01-03:02, 03:05-03:06	DRB1*03:35, DRB1*07:04, DRB1*14:38:01- 14:38:02, DRB1*14:50, DRB1*14:93, DRB1*14:127:01-14:127:02
34	180 bp 140 bp 175 bp	430 bp	*01:20 *03:05 *02:36	
35	250 bp	430 bp	*01:17, 02:33	
36⁵	105 bp 195 bp	430 bp	*01:22 *02:35	
37	155 bp	430 bp	*02:30	
38⁵	70 bp 205 bp	430 bp	*02:32 *01:07 [?] , 02:05 [?] , 02:07 [?] -02:08 [?] , 02:34	DRB1*03:44, DRB1*10:13, DRB1*13:106
39¹⁰	-	-	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 DRB3 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 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. In the presence of a specific amplification the intensity of the control band often decreases.

³For several 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.

⁴Due to the sharing of sequence motifs between DRB3 and DRB1 alleles, primer mixes 1 to 5, 7 to 17, 19, 20, 24, 26, 28, 29, 31 to 33 and 38 will amplify DRB1 alleles.

⁵HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁶Primer mixes 9, 10 and 13 may have tendencies of unspecific amplifications.

⁷Primer mix 2, 7, 9, 11, 19, 20 and 22 have a tendency to giving rise to primer oligomer formation.

⁸Primer mixes 4, 10 and 29 may give rise to a lower yield of HLA-specific PCR product than the other DRB3 primer mixes.

⁹Primer mix 33 gives rise to a notably lower yield of HLA-specific PCR product than the other DRB3 primer mixes in the DRB3*03:01:01-03:02 and 03:05-03:06 and in the DRB1*03:35, DRB1*14:38:01-14:38:02, 14:50, 14:93 and 14:127:01-14:127:02 alleles.

¹⁰Primer mix 39 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’, might be weakly amplified.

‘?’, nucleotide sequence information not available for the primer matching sequence.

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	100	125	95	240	95	190	90	120	165	185	270	90
PCR product					125		180					145
Length of int.	515	430	430	430	430	430	515	430	430	515	515	515
pos. control ¹												
5'-primer(s) ²	10(116) 5'-gCT 3'	29(175) 5'-gAT 3'	11(119) 5'-gCg 3'	114(429) 5'-CTg 3'	11(119) 5'-gCg 3'	8(110) 5'-CTC 3'	11(119) 5'-gCg 3'	11(119) 5'-gCg 3'	10(116) 5'-gCT 3'	29(175) 5'-gAC 3'	10(116) 5'-gCT 3'	51(239) 5'-gAg 3'
			11(119) 5'-gTg 3'						10(116) 5'-gCT 3'			
3'-primer(s) ³	29(175) 5'-gTA 3'	57(257) 5'-CgA 3'	28(171) 5'-CTg 3'	181(630) 5'-CTT 3'	28(171) 5'-CTC 3'	57(257) 5'-CgA 3'	27(169) 5'-gTT 3'	37(199) 5'-CAC 3'	51(239) 5'-CCC 3'	77(317) 5'-AgT 3'	86(344) 5'-CCA 3'	66(286) 5'-gAA 3'
	29(175) 5'-gTA 3'	57(257) 5'-CAA 3'	28(171) 5'-ATg 3'		38(202) 5'-gCT 3'		57(257) 5'-CAT 3'			77(318) 5'-TAA 3'		86(344) 5'-CCA 3'
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15	16	17	18	19	20	21	22	23	24
Length of spec.	270	185	120	150	120	100	180	170	120	185	195	185
PCR product		265	180		180	170	270		210	245	240	
			210									
Length of int.	515	515	430	430	430	430	515	430	430	430	430	430
pos. control ¹												
5'-primer(s) ²	10(116) 5'-gCT 3'	10(118) 5'-TgT 3'	11(119) 5'-gCT 3'	38(200) 5'-CgC 3'	10(116) 5'-gCT 3'	14(130) 5'-Agg 3'	10(116) 5'-gCT 3'	10(116) 5'-gCT 3'	10(116) 5'-gCT 3'	8(112) 5'-TgC 3'	10(118) 5'-TgT 3'	11(119) 5'-gCT 3'
	10(116) 5'-gCT 3'	38(200) 5'-CgC 3'				38(200) 5'-CgC 3'				10(118) 5'-TgT 3'	25(163) 5'-ggC 3'	
		38(200) 5'-CgC 3'								29(173) 5'-CAT 3'		
3'-primer(s) ³	86(344) 5'-CAC 3'	86(344) 5'-CAC 3'	37(197) 5'-Cgg 3'	74(308) 5'-CCC 3'	36(196) 5'-gTT 3'	57(257) 5'-CgA 3'	55(251) 5'-gCA 3'	51(239) 5'-CCg 3'	36(196) 5'-gAg 3'	77(318) 5'-TAA 3'	77(318) 5'-TAA 3'	58(260) 5'-CCT 3'
			37(197) 5'-CgA 3'		36(196) 5'-gTT 3'		56(256) 5'-gCT 3'	57(257) 5'-Cag 3'	66(286) 5'-gAT 3'			60(266) 5'-Agg 3'
			57(257) 5'-CAg 3'		55(251) 5'-gCA 3'		58(260) 5'-CCT 3'		69(294) 5'-TgA 3'			
			66(286) 5'-gAA 3'		57(257) 5'-CAg 3'		86(344) 5'-CAg 3'					
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

101.121-24/04 – including *Taq* pol., IFU-01
101.121-24u/04u – without *Taq* pol., IFU-02

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Lot No.: **2E8**

Lot-specific information

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Well No.	25	26	27	28	29	30	31	32	33	34	35	36
Length of spec.	225	235	90	235	160	210	70	230	150	140	250	105
PCR product	260			260			95		180	175		195
Length of int.	430	430	430	430	430	430	430	430	430	430	430	430
pos. control ¹												
5'-primer(s) ²	10(116) 5'-gCT 3'	12(122) 5'-TAC 3'	163(577) 5'-CAT 3'	10(116) 5'-gCT 3'	38(200) 5'-CgC 3'	123(455) 5'-CTg 3'	51(239) 5'-gAg 3'	11(119) 5'-gCT 3'	37(197) 5'-gTT 3'	33(186) 5'-AAG 3'	10(116) 5'-gCT 3'	10(116) 5'-gCT 3'
							149(534) 5'-CAg 3'			43(217) 5'-ACA 3'		
3'-primer(s) ³	69(294) 5'-TgA 3'	77(317) 5'-AAT 3'	179(624) 5'-ACg 3'	74(308) 5'-CCg 3'	77(317) 5'-Agg 3'	179(624) 5'-ACg 3'	60(266) 5'-Agg 3'	74(308) 5'-CCC 3'	74(308) 5'-CCT 3'	77(318) 5'-TAA 3'	76(316) 5'-ATC 3'	31(180) 5'-TgC 3'
	71(299) 5'-gCC 3'			83(337) 5'-CCg 3'			165(583) 5'-CCg 3'		84(338) 5'-CCA 3'		81(329) 5'-Tgg 3'	61(270) 5'-TTA 3'
	83(337) 5'-CCg 3'						169(595) 5'-AAA 3'					
Well No.	25	26	27	28	29	30	31	32	33	34	35	36

Well No.	37	38
Length of spec.	155	70
PCR product		205
Length of int.	430	430
pos. control ¹		
5'-primer(s) ²	10(116) 5'-gCT 3'	38(200) 5'-CgC 3'
3'-primer(s) ³	48(230) 5'-CCg 3'	47(227) 5'-ggA 3'
		93(365) 5'-gCg 3'
Well No.	37	38

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 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.

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

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

101.121-24/04 – including *Taq* pol., IFU-01
101.121-24u/04u – without *Taq* pol., IFU-02

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Lot No.: **2E8**

Lot-specific information

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CELL LINE VALIDATION SHEET																					
DRB3 SSP subtyping kit ²																					
					Well ³																
						1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.	201555801	201555802	201555803	201555804	201555805	201555806	201555807	201555808	201555809	201671210	201555811	201555812	201555813	201555814	201555815	201555816	
	IHWC cell line ¹		DRB3																		
1	9001	SA			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*02:02		-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
5	9009	KAS011			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*02:02		-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
8	9025	DEU			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*02:02		-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
17	9282	CTM3953540	*01:01		+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
18	9257	32367	*02:02		-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
19	9038	BM16	*02:02		-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
20	9059	SLE005	*03:01		+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-
21	9064	AMALA	*01:01		+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
22	9056	KOSE	*02:02	*03:01	+	+	-	+	-	-	-	-	-	+	+	+	-	+	+	+	-
23	9124	IHL	*02:02		-	-	-	+	-	-	-	-	-	+	+	-	-	+	+	-	-
24	9035	JBUSH	*02:02		-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
25	9049	IBW9			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*02:02		-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
27	9191	CH1007			-	-	-	-	-	-	-	-	-	-	-	-	-	-	W	-	-
28	9320	BEL5GB			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*01:01		+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
31	9019	DUCAF	*02:02		-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
32	9297	HAG	*01:01		+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
33	9098	MT14B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*02:02		-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
35	9302	SSTO			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*01:01		+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
38	9099	LZL	*01:01		+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
39	9315	CML	*01:01		+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
40	9134	WHONP199			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*03:01		+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-
42	9066	TAB089			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*02:24		-	-	-	+	-	-	-	-	-	+	+	+	+	-	-	-	-
45	9239	SHJO			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*02:02		-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
48	9303	TER-ND			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

101.121-24/04 – including *Taq* pol., IFU-01
101.121-24u/04u – without *Taq* pol., IFU-02

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Lot No.: **2E8**

Lot-specific information

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CELL LINE VALIDATION SHEET																																
DRB3 SSP subtyping kit ²																																
					Prod. No.	Well ³																										
						17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32											
						201555817	201671218	201555819	201555820	201671221	201555822	201555823	201555824	201671225	201555826	201555827	201671228	201555829	201555830	201555831	201671232											
	IHWC cell line ¹		DRB3																													
1	9001	SA				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2	9280	LK707				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
3	9011	E4181324				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4	9275	GU373	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
5	9009	KAS011				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
6	9353	SM				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
7	9020	QBL	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
8	9025	DEU				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
9	9026	YAR				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
10	9107	LKT3				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
11	9051	PITOUT				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
12	9052	DBB				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
13	9004	JESTHOM				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
14	9071	OLGA				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
15	9075	DKB				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
16	9037	SWEIG007	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
17	9282	CTM3953540	*01:01			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
18	9257	32367	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
19	9038	BM16	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
20	9059	SLE005	*03:01			-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
21	9064	AMALA	*01:01			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
22	9056	KOSE	*02:02	*03:01		-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
23	9124	IHL	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
24	9035	JBUSH	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
25	9049	IBW9				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
26	9285	WT49	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
27	9191	CH1007				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
28	9320	BEL5GB				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
29	9050	MOU				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
30	9021	RSH	*01:01			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
31	9019	DUCAF	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
32	9297	HAG	*01:01			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
33	9098	MT14B				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
34	9104	DHIF	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
35	9302	SSTO				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
36	9024	KT17				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
37	9065	HHKB	*01:01			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
38	9099	LZL	*01:01			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
39	9315	CML	*01:01			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
40	9134	WHONP199				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
41	9055	H0301	*03:01			-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
42	9066	TAB089				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
43	9076	T7526				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
44	9057	TEM	*02:24			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
45	9239	SHJO				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
46	9013	SCHU				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
47	9045	TUBO	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
48	9303	TER-ND				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

101.121-24/04 – including *Taq* pol., IFU-01
101.121-24u/04u – without *Taq* pol., IFU-02

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Lot No.: **2E8**

Lot-specific information

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CELL LINE VALIDATION SHEET											
DRB3 SSP subtyping kit ²											
					Prod. No.	Well ³					
						33	34	35	36	37	38
						201671233	201671234	201671235	201671236	201671237	201671238
	IHWC cell line ¹	DRB3									
1	9001 SA				-	-	-	-	-	-	-
2	9280 LK707				-	-	-	-	-	-	-
3	9011 E4181324				-	-	-	-	-	-	-
4	9275 GU373	*02:02			-	-	-	-	-	-	-
5	9009 KAS011				-	-	-	-	-	-	-
6	9353 SM				-	-	-	-	-	-	-
7	9020 QBL	*02:02			-	-	-	-	-	-	-
8	9025 DEU				-	-	-	-	-	-	-
9	9026 YAR				-	-	-	-	-	-	-
10	9107 LKT3				-	-	-	-	-	-	-
11	9051 PITOUT				-	-	-	-	-	-	-
12	9052 DBB				-	-	-	-	-	-	-
13	9004 JESTHOM				-	-	-	-	-	-	-
14	9071 OLGA				-	-	-	-	-	-	-
15	9075 DKB				-	-	-	-	-	-	-
16	9037 SWEIG007	*02:02			-	-	-	-	-	-	-
17	9282 CTM3953540	*01:01			-	-	-	-	-	-	-
18	9257 32367	*02:02			-	-	-	-	-	-	-
19	9038 BM16	*02:02			-	-	-	-	-	-	-
20	9059 SLE005	*03:01			+	-	-	-	-	-	-
21	9064 AMALA	*01:01			-	-	-	-	-	-	-
22	9056 KOSE	*02:02	*03:01		+	-	-	-	-	-	-
23	9124 IHL	*02:02			-	-	-	-	-	-	-
24	9035 JBUSH	*02:02			-	-	-	-	-	-	-
25	9049 IBW9				-	-	-	-	-	-	-
26	9285 WT49	*02:02			-	-	-	-	-	-	-
27	9191 CH1007				-	-	-	-	-	-	-
28	9320 BEL5GB				-	-	-	-	-	-	-
29	9050 MOU				-	-	-	-	-	-	-
30	9021 RSH	*01:01			-	-	-	-	-	-	-
31	9019 DUCAF	*02:02			-	-	-	-	-	-	-
32	9297 HAG	*01:01			-	-	-	-	-	-	-
33	9098 MT14B				-	-	-	-	-	-	-
34	9104 DHIF	*02:02			-	-	-	-	-	-	-
35	9302 SSTS				-	-	-	-	-	-	-
36	9024 KT17				-	-	-	-	-	-	-
37	9065 HHKB	*01:01			-	-	-	-	-	-	-
38	9099 LZL	*01:01			-	-	-	-	-	-	-
39	9315 CML	*01:01			-	-	-	-	-	-	-
40	9134 WHONP199				-	-	-	-	-	-	-
41	9055 H0301	*03:01			+	-	-	-	-	-	-
42	9066 TAB089				-	-	-	-	-	-	-
43	9076 T7526				-	-	-	-	-	-	-
44	9057 TEM	*02:24			-	-	-	-	-	-	-
45	9239 SHJO				-	-	-	-	-	-	-
46	9013 SCHU				-	-	-	-	-	-	-
47	9045 TUBO	*02:02			-	-	-	-	-	-	-
48	9303 TER-ND				-	-	-	-	-	-	-

101.121-24/04 – including *Taq* pol., IFU-01
101.121-24u/04u – without *Taq* pol., IFU-02

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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.

³Cell line TEM, IHW 9057, is profiled as DRB3*02:01 by ECACC ((European Collection of Cell Cultures)). However, Olerup SSP AB has shown that this cell line includes the exon 3 polymorphism corresponding to DRB3*02:24. Primer mix 4 amplifies DRB1*14 alleles in the KOSE, IHL and TEM cell lines. The DRB1*10:01 allele is weakly amplified by primer mix 14 in the CH1007 cell line.

No DNAs carrying the alleles to be amplified by primer solutions 5 to 8, 16 to 19, 21 to 23, 25 to 32 and 34 to 38 were available. The specificities of the primers in primer solutions 5, 7, 8, 16 to 19, 21, 22, 25, 26, 28, 29, 31, 32 and 38 were tested by separately adding additional 5'-primers, respectively additional 3'-primers.

In primer solutions 6, 23, 27, 30 and 34 it was only possible to test the 3'-primer, the 5'-primers were not possible to test. In primer solutions 35 to 37 it was only possible to test the 5'-primer, the 3'-primers were not possible to test. One or two of the 3'-primers in primer solutions 1 to 3, 5, 7, 12, 15, 17, 19, 21, 25, 28, 31, 33 and 38 were not possible to test. In primer solutions 2, 3, 14, 18 and 22 one or two of the 5'-primers were not possible to test. Additional 3'-primers in primer solutions 15, 20 and 24 were tested by separately adding one 5'-primer.

101.121-24/04 – including *Taq* pol., IFU-01
101.121-24u/04u – without *Taq* pol., IFU-02

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