

101.519-12 – including *Taq* pol., IFU-01
101.519-12u – without *Taq* pol., IFU-02

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

Lot No.: **5D1**

Lot-specific information
Olerup SSP® HLA-B*18

Product number:	101.519-12 – including <i>Taq</i> polymerase 101.519-12u – without <i>Taq</i> polymerase
Lot number:	5D1
Expiry date:	2018-08-01
Number of tests:	12
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. 5D1.

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*18 Lot (56X)**

The HLA-B*18 kit is updated for new alleles to enable separation of:

- Confirmed¹ alleles as listed in the IMGT/HLA database
- Polymorphisms in exons outside of the region encoding the peptide binding domain
- Null and Alternatively expressed alleles

A well containing Negative Control primer pairs has been added.

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

¹As described in section Uniquely Identified Alleles.

The HLA-B*18 primer set, specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP®* HLA-B*18 lot was made (**Lot No. 56X**). The kit design is based on IMGT/HLA database 3.22.0.

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
1	-	Added	3'-primer added for the B*18:01:23 allele.
6	-	Added	3'-primer added for the B*18:60 allele.
14	Added	-	5'-primer added for the B*18:102 allele.
17	Moved, added	-	5'-primers moved to wells 29 and 30, 5'-primer added for the B*18:69 allele.
18	Exchanged	-	5'-primer exchanged for improved HLA-specific amplification.
24	Exchanged	Exchanged	Primer pair exchanged for improved HLA-specific amplification.
25	-	Modified, added	3'-primer modified for improved HLA-specific amplification, 3'-primer added for the B*18:95 allele.
26	Added	-	5'-primer added for the B*18:92 allele.
27	-	Added	3'-primer added for the B*18:94N allele.
28	-	Added	3'-primer added for the B*18:95 allele.
29	Added	-	5'-primer added from well 17 for the B*18:18 allele.
30	Added	-	5'-primer added from well 17 for the B*18:31 allele.

Changes in revision R01 compared to R00:

1. Primer mix 11 does not amplify the B*18:10-18:11, 18:21, 18:35 and 18:110 and the B*39:39:02 alleles. This has been corrected in the Specificity and Interpretation Tables. Furthermore, primer mix 11 does not amplify the B*18:56, 18:58 and 18:101 and the B*15:01:38, 15:64:02, 35:02:02, 35:20:02, 44:03:12, A*24:02:37 and A*26:01:16 alleles with the HLA-specific PCR product of 635 bp. This has been corrected in the Specificity Table and in the foot notes to the Interpretation 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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Lot-specific information

PRODUCT DESCRIPTION

HLA-B*18 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*18:01 to B*18:120 alleles.

PLATE LAYOUT

Each test consists of 32 PCR reactions in a 32 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	24
25	26	27	28	29	30	31	NC

The 32 well cut PCR plate is marked with 'HLA-B*18' in silver/gray ink.

Well No. 1 is marked with the Lot No. '5D1'.

Wells 1 to 31 – HLA-B*18 high resolution primers.

Well 32 – 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 32 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 non-HLA-B*18 alleles will be amplified by primer mixes 1 to 5, 7, 8, 10 to 18, 20 to 22, 24 to 31. In addition, a few HLA-A and HLA-C alleles will be amplified by primer mixes 2, 8, 9, 11, 13, 15, 16, 18, 21, 22, 26, 27 and 30.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*18 alleles, i.e. **B*18:01 to B*18:120**, recognized by the HLA Nomenclature Committee in October 2015 will be amplified by the primers in the HLA-B*18 subtyping kit^{1,2}.

The HLA-B*18 kit enables separation of the confirmed HLA-B*18 alleles as listed in the IMGT/HLA database. An HLA allele is listed as confirmed by IMGT/HLA if it has been sequenced by more than a single laboratory or from multiple sources. Current allele confirmation status for HLA-B*18 alleles is listed below.

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The HLA-B*18 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.

The following HLA-B*18 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
B*18:05, 18:60	6	B*18:36, 18:67	11
B*18:18, 18:47	29	B*18:37, 18:46	27
B*18:23N, 18:28	10	B*18:38, 18:45	28
B*18:32, 18:98	18	B*18:40, 18:51	23
B*18:34, 18:52	30	B*18:42, 18:43	26

The HLA-B*18 kit cannot distinguish the silent mutations in the B*18:01:01:01-18:01:23, the B*18:07:01-18:07:02, the B*18:12:01-18:12:02 or the B*18:44:01-18:44:02 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2015-October-10, release 3.22.0, 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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ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
B*18:01:01:01	Confirmed	B*18:17N	Confirmed	B*18:56	Unconfirmed	B*18:96	Unconfirmed
B*18:01:01:02	Confirmed	B*18:18	Unconfirmed	B*18:57	Unconfirmed	B*18:97	Unconfirmed
B*18:01:02	Unconfirmed	B*18:19	Confirmed	B*18:58	Unconfirmed	B*18:98	Unconfirmed
B*18:01:03	Unconfirmed	B*18:20	Confirmed	B*18:59	Unconfirmed	B*18:99	Unconfirmed
B*18:01:04	Unconfirmed	B*18:21	Unconfirmed	B*18:60	Confirmed	B*18:100	Unconfirmed
B*18:01:05	Confirmed	B*18:22	Confirmed	B*18:61	Confirmed	B*18:101	Unconfirmed
B*18:01:06	Confirmed	B*18:23N	Unconfirmed	B*18:62	Unconfirmed	B*18:102	Unconfirmed
B*18:01:07	Unconfirmed	B*18:24	Unconfirmed	B*18:63	Unconfirmed	B*18:103	Unconfirmed
B*18:01:08	Confirmed	B*18:25	Confirmed	B*18:64	Unconfirmed	B*18:104	Unconfirmed
B*18:01:09	Unconfirmed	B*18:26	Confirmed	B*18:65	Unconfirmed	B*18:105	Unconfirmed
B*18:01:10	Confirmed	B*18:27	Unconfirmed	B*18:66	Unconfirmed	B*18:106	Unconfirmed
B*18:01:11	Unconfirmed	B*18:28	Confirmed	B*18:67	Unconfirmed	B*18:107	Unconfirmed
B*18:01:12	Unconfirmed	B*18:29	Unconfirmed	B*18:68	Confirmed	B*18:108	Unconfirmed
B*18:01:13	Unconfirmed	B*18:30	Unconfirmed	B*18:69	Confirmed	B*18:109	Unconfirmed
B*18:01:14	Unconfirmed	B*18:31	Unconfirmed	B*18:70	Unconfirmed	B*18:110	Unconfirmed
B*18:01:15	Unconfirmed	B*18:32	Confirmed	B*18:71	Unconfirmed	B*18:111	Unconfirmed
B*18:01:16	Unconfirmed	B*18:33	Confirmed	B*18:72	Unconfirmed	B*18:112	Unconfirmed
B*18:01:17	Unconfirmed	B*18:34	Confirmed	B*18:73	Confirmed	B*18:113	Unconfirmed
B*18:01:18	Unconfirmed	B*18:35	Confirmed	B*18:74N	Unconfirmed	B*18:114	Unconfirmed
B*18:01:19	Unconfirmed	B*18:36	Unconfirmed	B*18:75	Confirmed	B*18:115	Unconfirmed
B*18:01:20	Unconfirmed	B*18:37	Confirmed	B*18:76	Unconfirmed	B*18:116	Unconfirmed
B*18:01:21	Unconfirmed	B*18:38	Unconfirmed	B*18:77	Confirmed		
B*18:01:22	Confirmed	B*18:39	Confirmed	B*18:78	Unconfirmed		
B*18:01:23	Unconfirmed	B*18:40	Confirmed	B*18:79	Confirmed		
B*18:02	Confirmed	B*18:41	Unconfirmed	B*18:80	Unconfirmed		
B*18:03	Confirmed	B*18:42	Unconfirmed	B*18:81	Unconfirmed		
B*18:04	Confirmed	B*18:43	Confirmed	B*18:82	Unconfirmed		
B*18:05	Confirmed	B*18:44:01	Confirmed	B*18:83	Unconfirmed		
B*18:06	Confirmed	B*18:44:02	Unconfirmed	B*18:84	Unconfirmed		
B*18:07:01	Confirmed	B*18:45	Confirmed	B*18:85	Unconfirmed		
B*18:07:02	Confirmed	B*18:46	Unconfirmed	B*18:86	Unconfirmed		
B*18:08	Confirmed	B*18:47	Confirmed	B*18:87	Unconfirmed		
B*18:09	Confirmed	B*18:48	Unconfirmed	B*18:88	Unconfirmed		
B*18:10	Unconfirmed	B*18:49	Confirmed	B*18:89	Unconfirmed		
B*18:11	Confirmed	B*18:50	Confirmed	B*18:90	Unconfirmed		
B*18:12:01	Confirmed	B*18:51	Confirmed	B*18:91	Unconfirmed		
B*18:12:02	Unconfirmed	B*18:52	Unconfirmed	B*18:92	Unconfirmed		
B*18:13	Confirmed	B*18:53	Confirmed	B*18:93	Unconfirmed		
B*18:14	Confirmed	B*18:54	Confirmed	B*18:94N	Confirmed		
B*18:15	Unconfirmed	B*18:55	Unconfirmed	B*18:95	Confirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2015-October-10, release 3.22.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-B*18 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

HLA-B*18 SSP subtyping

Specificities and sizes of the PCR products of the 31+1 primer mixes used
for HLA-B*18 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*18 alleles ³	Other amplified HLA Class I alleles ⁴
1	170 bp	800 bp	*18:01:01:01-18:02, 18:04-18:08, 18:10-18:15, 18:17N-18:28, 18:30-18:53, 18:55-18:71, 18:73-18:91, 18:93, 18:95-18:101, 18:103-18:106, 18:109-18:116	*35:289, 48:28
2	260 bp	800 bp	*18:01:01:01-18:01:23, 18:03-18:09, 18:12:01-18:13, 18:15, 18:17N-18:20, 18:23N-18:26, 18:28-18:34, 18:36-18:38, 18:40-18:43, 18:45-18:55, 18:57, 18:59-18:68, 18:70-18:100, 18:102-18:104, 18:106-18:109, 18:111-18:116	*14:06:01-14:06:02, 14:08:01-14:08:02, 15:37-15:38:02, 15:185, 15:323, 15:335, 15:364, 15:368, 39:32, 39:43, 39:48, 40:51, 40:113, 40:163, 51:06:01-51:06:03, 51:45, 51:62, 51:135, 51:194, 56:31, C*08:51, C*08:114
3	260 bp	800 bp	*18:02	*27:28, 27:62
4⁵	100 bp	800 bp	*18:03	*39:39:02
5⁵	115 bp	1070 bp	*18:72	*15:01:38 ^w , 15:64:02, 35:02:02 ^w , 35:20:02 ^w , 39:39:02
	145 bp		*18:04, 18:24	
6	130 bp 245 bp	1070 bp	*18:05 *18:60	
7⁵	90 bp 150 bp 230 bp	1070 bp	*18:41 *18:07:01-18:07:02, 18:79 *18:74N	*35:289
8	180 bp	1070 bp	*18:10, 18:21, 18:35, 18:50, 18:81	*07:02:01-07:04, 07:07-07:13, 07:15-07:16, 07:18:01-07:18:02, 07:21-07:23, 07:25-07:27, 07:29-07:30, 07:33:01-07:33:03, 07:35-07:39, 07:41-07:42, 07:44-07:52, 07:54-07:59, 07:61-07:68:03, 07:70-07:77, 07:79, 07:81-07:89, 07:91-07:95, 07:98-07:99, 07:101-07:104, 07:106-07:111N, 07:113-07:114, 07:116-07:122, 07:124-07:130, 07:132-07:136:02, 07:139, 07:141-07:175, 07:177-07:181N, 07:183-07:196, 07:198-07:200, 07:202-07:205, 07:208, 07:211-07:212, 07:215-07:219, 07:221, 07:224, 07:226-07:234, 07:236-07:248, 07:250-07:256, 08:156, 13:26:01-13:26:02, 13:35, 13:72, 15:40, 15:47:01-15:47:02, 15:49, 15:52, 15:114, 15:117, 15:124, 15:138, 15:214, 15:238, 15:241, 27:34, 27:41, 27:136, 35:15:01-35:15:02, 35:33,

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				35:66, 35:86, 35:250, 37:07, 40:03, 40:18, 40:20, 40:24, 40:31-40:33, 40:44, 40:52, 40:59, 40:98, 40:120, 40:150, 40:159, 40:267, 40:284, 46:06, 46:25, 46:30, 48:08, 48:23, 51:165, 57:13, 57:25, 57:29, 58:37, C*02:02:19, C*02:06, C*02:23, C*02:36, C*15:42, C*15:111
9⁵	125 bp 255 bp	1070 bp	*18:75 *18:08, 18:27	A*24:220
10^{5,7}	85 bp 175 bp	800 bp	*18:23N *18:28	*44:108N *15:250, 27:132, 40:226
11⁵	125 bp 610 bp 635 bp	1070 bp	*18:09, 18:54, 18:67 *18:36, 18:56-18:58, 18:101	*44:03:12, A*24:02:37 *15:01:38, 15:64:02, 35:02:02, 35:20:02, 44:03:12, A*26:01:16
12⁵	80 bp 140 bp	1070 bp	*18:41, 18:73 *18:12:01-18:12:02, 18:77	*48:28
13⁵	85 bp 265 bp	1070 bp	*18:13, 18:19, 18:30 *18:44:01-18:44:02	*07:218, 07:220, 14:03, 15:38:01-15:38:02, 15:368, 51:29, 51:61:01-51:61:02, 51:82, 52:21, 52:24, A*66:04^w, C*01:30, C*08:51, C*08:114, C*12:87 *14:01:01-14:02:08, 14:02:10-14:04, 14:07N, 14:09, 14:11-14:12, 14:14-14:36, 14:38-14:45, 14:47-14:50, C*01:30, C*12:87
14⁵	110 bp 170 bp 260 bp	1070 bp	*18:102, 18:107 *18:04, 18:25, 18:68, 18:107 *18:14	*44:03:12 *15:01:38, 15:64:02, 35:02:02, 35:20:02, 44:03:12 *07:218, 07:220, 08:118, 14:05, 14:13, 40:174, 51:64
15^{5,6}	100 bp	1070 bp	*18:15, 18:19, 18:30-18:31, 18:57	*07:218, 13:62, 14:01:01-14:40, 14:42, 14:44-14:45, 14:47-14:50, 15:37-15:38:02, 15:323, 15:335, 15:364, 15:368, 35:21, 35:96, 35:188, 40:28, 40:51, 40:174, 49:09, 51:01:01:01-51:01:53, 51:03-51:04, 51:06:01-51:07:02, 51:11N-51:14, 51:16-51:18, 51:21-51:22, 51:24:01-51:24:05, 51:26-51:30, 51:32-51:33, 51:35, 51:37-51:39, 51:41N, 51:43, 51:45-51:46, 51:48-51:52, 51:55-51:58, 51:60-51:72, 51:74-51:80, 51:82-51:92:02, 51:94-51:96, 51:98N-51:107, 51:109-51:114, 51:116-51:119, 51:121, 51:123-51:138, 51:140, 51:142, 51:145, 51:147, 51:149N-51:152, 51:154-51:156, 51:158:01-51:174, 51:176-51:179, 51:182-51:193, 51:195, 52:01:01:01-52:02:02, 52:04-52:09, 52:12-52:18, 52:20-52:28, 52:30-52:56, 53:06, 53:28, 56:05:01-56:06, 56:21, 58:08:01-58:08:02, 78:01:01-78:03, 78:05-78:07, 78:09, C*01:30, C*08:51, C*08:114, C*12:87, C*15:39
16⁵	95 bp	1070 bp	*18:19	*08:70, 13:31, 13:41, 15:07:03, 15:27:03, 15:38:02, 15:269, 35:14:01, 35:62, 35:163, 39:36, 44:146, 46:01:03, 46:01:16, 51:61:02, 52:21, 54:33, 55:21, 56:43, A*26:68,

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				C*01:09, C*02:02:14, C*06:02:27, C*07:22, C*07:138, C*12:03:16, C*12:03:30
	360 bp		*18:17N	
17⁶	270 bp	1070 bp	*18:69	*37:04:01-37:04:02
18^{5,6}	85 bp	1070 bp	*18:29, 18:98	*15:71, 15:175, 15:204, 15:225, 15:344, 35:50, 35:231, 35:280, 37:22, 40:248, 40:282, 41:12, 41:36, 46:14, 49:03, 51:151, 52:51, 54:02, 55:16, 56:35, 57:39, 57:73, 58:41, C*03:278, C*16:86
	175 bp		*18:32	*15:192, 15:281
	200 bp		*18:15, 18:21 ^w , 18:30 ^w , 18:57, 18:101	*07:07 ^w , 07:214 ^w , 07:255 ^w , 14:06:01, 14:08:02, 15:03:01:01-15:03:05, 15:09- 15:10:04, 15:17:01:01-15:18:01, 15:18:03- 15:18:06, 15:23, 15:29, 15:37, 15:39:01- 15:40, 15:47:01 ^w -15:47:02 ^w , 15:49 ^w , 15:61, 15:64:01-15:64:02, 15:65 ^w , 15:69, 15:72, 15:74, 15:90, 15:93, 15:98, 15:99 ^w , 15:103, 15:108, 15:115, 15:119, 15:127, 15:131- 15:134, 15:138, 15:153, 15:156, 15:158, 15:161, 15:168 ^w , 15:173, 15:176-15:177, 15:196, 15:198, 15:208, 15:210, 15:216, 15:219-15:220, 15:221 ^w , 15:226N, 15:229- 15:230, 15:238, 15:242-15:243, 15:252- 15:253, 15:255, 15:263, 15:266, 15:268, 15:273-15:274, 15:281-15:282, 15:288, 15:292, 15:294N, 15:306-15:307, 15:311- 15:314, 15:323, 15:335, 15:337-15:339, 15:351, 15:354, 15:356, 15:361, 15:364, 15:369, 27:21, 35:99 ^w , 38:10, 38:32, 39:35, 39:107, 40:23 ^w , 40:26, 40:51, 40:158, 40:183, 40:198 ^w , 46:03 ^w , 46:08, 46:62 ^w , 51:06:01, 51:06:03, 51:45, 51:59, 51:62, 51:135, 56:32, C*01:79:01^w, C*02:16:01- 02:16:02, C*02:32^w, C*03:13:02, C*03:135, C*04:15:01^w, C*04:15:03^w, C*05:01:03^w, C*05:22:02^w, C*12:18:01-12:18:02, C*12:83, C*12:85^w, C*14:09^w
19⁵	105 bp 160 bp 405 bp	1070 bp	*18:06 *18:53 *18:39	
20⁶	290 bp	1070 bp	*18:20	*07:14, 07:17, 07:28, 07:96:01-07:96:02, 07:115, 07:197, 07:225, 08:14, 08:28, 08:49, 08:74, 08:87, 08:111, 13:10, 13:46, 13:51, 13:79, 14:01:01-14:12, 14:14-14:22, 14:24- 14:50, 15:02:01-15:02:09, 15:06, 15:13:01- 15:13:02, 15:17:01:01-15:17:05, 15:21, 15:25:01-15:25:03, 15:31, 15:36, 15:44, 15:55, 15:77, 15:88-15:89, 15:106, 15:112, 15:121, 15:128, 15:137, 15:139, 15:144, 15:156, 15:162, 15:168, 15:170, 15:177, 15:196, 15:204, 15:208, 15:213-15:214, 15:216, 15:230, 15:233, 15:240, 15:250, 15:265, 15:271, 15:273, 15:283, 15:289, 15:291, 15:297, 15:301-15:302N, 15:308,

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				15:319, 15:325, 15:328, 15:330, 15:339, 15:341-15:342, 15:345, 15:356-15:358, 15:360, 15:366, 15:374, 27:01-27:06, 27:08-27:10, 27:12-27:19, 27:21, 27:23, 27:25-27:26, 27:28-27:32, 27:35-27:40, 27:42, 27:44-27:104, 27:106-27:124, 27:126-27:129, 27:131-27:135, 27:137-27:145, 35:164, 35:215, 35:234, 35:261, 37:01:01-37:06:02, 37:08, 37:10-37:11, 37:13-37:34, 37:36-37:55, 37:57, 38:22, 39:17, 40:09, 40:42, 40:60, 40:89, 40:124:01-40:124:02, 40:172, 40:180, 40:280, 42:20, 44:02:01:01-44:03:21, 44:03:23-44:09, 44:11-44:13, 44:15-44:16, 44:18-44:30, 44:32-44:42, 44:44, 44:46-44:50:02, 44:52N-44:61N, 44:63-44:76, 44:78-44:81, 44:83-44:106, 44:108N-44:121, 44:124-44:134, 44:136-44:165, 44:167-44:183, 44:185-44:212, 44:214-44:229, 44:231-44:234, 45:01:01-45:08, 45:10-45:13, 45:15-45:17, 47:01:01:01-47:10, 48:17, 48:26, 49:01:01-49:03, 49:05-49:14, 49:16-49:35, 49:37-49:40, 50:01:01-50:02, 50:04-50:20, 50:31-50:43, 51:42, 51:85, 51:172, 52:26, 53:07, 54:15, 55:41, 55:58, 57:04:01-57:05, 57:32, 57:41, 83:01
21	215 bp	1070 bp	*18:01:01:01-18:09, 18:12:01-18:15, 18:17N-18:19, 18:22-18:34, 18:36-18:55, 18:57, 18:59-18:100, 18:102, 18:104-18:109, 18:111-18:116	*07:218, 07:220, 08:118, 13:15, 13:62, 14:13, 14:23, 15:37-15:38:02, 15:185, 15:323, 15:335, 15:364, 15:368, 35:21, 35:24:01-35:24:02, 35:81, 35:96, 35:109, 35:157, 35:188, 35:190, 35:233, 35:287, 39:32, 39:43, 39:48, 40:28, 40:51, 40:113, 40:163, 40:174, 51:01:01:01-51:01:49, 51:01:51-51:01:53, 51:03-51:04, 51:06:01-51:09:03, 51:11N-51:14, 51:16, 51:19-51:22, 51:24:01-51:24:05, 51:26-51:33, 51:35, 51:37-51:39, 51:41N, 51:43-51:46, 51:48-51:53, 51:55-51:58, 51:60-51:80, 51:82-51:84, 51:86-51:92:02, 51:94-51:107, 51:109-51:142, 51:145, 51:147, 51:149N-51:156, 51:158:01-51:171, 51:173Q-51:174, 51:176-51:179, 51:181-51:195, 52:01:01:01-52:02:02, 52:04-52:10:03, 52:12-52:25, 52:27-52:56, 53:02, 53:06, 53:28, 56:05:01-56:06, 56:21, 56:31, 56:34, 57:14:01-57:14:02, 58:08:01-58:09, 78:01:01-78:03, 78:05-78:09, C*15:39
22	130 bp 150 bp 270 bp	800 bp	*18:75, 18:77 *18:33 *18:22, 18:69 ^w , 18:105	*14:10, 35:21, 35:24:01-35:24:02, 35:81, 35:96, 35:109, 35:157, 35:188, 35:190, 35:233, 35:287, 37:04:01 ^w -37:04:02 ^w , 40:28, 44:190, 44:203, 51:04, 51:46, 51:56:01-51:56:03, 51:139, 53:02, 53:06, 53:28, 57:14:01-57:14:02, 58:09, C*15:39

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Lot-specific information

23	145 bp 160 bp 515 bp	1070 bp	*18:51 *18:53 *18:21, 18:40, 18:61	
24	205 bp	1070 bp	*18:26, 18:61	*07:218, 07:220, 08:118, 13:62, 14:13, 14:23, 15:37, 15:323, 35:81, 35:157, 35:233, 40:28, 40:51, 40:113, 40:163, 40:174, 51:01:01:01-51:01:14, 51:01:16-51:01:53, 51:03-51:04, 51:06:01-51:09:03, 51:11N- 51:12, 51:16, 51:18-51:22, 51:24:01- 51:24:05, 51:26-51:33, 51:35, 51:38-51:39, 51:41N, 51:43-51:44N, 51:46, 51:48-51:53, 51:55-51:58, 51:60-51:61:02, 51:64-51:80, 51:82-51:84, 51:86-51:91, 51:94-51:96, 51:98N-51:105, 51:107, 51:109-51:142, 51:145, 51:147, 51:149N-51:156, 51:158:01- 51:171, 51:173Q-51:174, 51:176-51:179, 51:181, 51:183-51:191, 51:193-51:195, 52:01:01:01-52:02:02, 52:04-52:10:03, 52:12-52:13, 52:15-52:24, 52:27-52:38, 52:40-52:56, 56:05:01-56:06, 56:21, 58:08:01-58:08:02, 78:01:01-78:03, 78:05- 78:09
25⁵	75 bp 190 bp	1070 bp	*18:48 *18:35, 18:54, 18:95, 18:110	*07:68:01-07:68:03, 07:214, 07:237, 15:138, 15:230, 15:241, 15:297, 35:66, 40:77, 40:87:01-40:87:02, 40:121, 40:158, 40:222, 40:237, 44:150, 48:12, 48:14, 48:26, 48:29
26^{5,7}	90 bp 115 bp 180 bp	1070 bp	*18:42 *18:92 *18:04, 18:25, 18:43, 18:107	*15:01:38, 15:64:02, 35:02:02, 35:20:02, 39:39:02, 44:03:12, A*24:02:37, A*26:01:16
27⁵	80 bp 135 bp 175 bp	800 bp	*18:37, 18:73, *18:46, 18:74N, 18:78 *18:94N	*07:109, 48:28 *13:10, 13:26:01-13:26:02, 13:72, C*12:120
28⁵	100 bp 175 bp	800 bp	*18:45 *18:38, 18:74N, 18:78	*08:83, 13:10-13:11, 13:71, 38:38, 40:91, 40:197
	200 bp		*18:95	
29^{5,8}	65 bp	1070 bp	*18:50	*07:218, 13:15, 40:113, 51:16, 51:31, 51:82, 52:08, 73:01, 78:08
	120 bp 255 bp		*18:47 *18:18	
30^{5,6}	100 bp 145 bp 420 bp 495 bp	800 bp	*18:31 *18:34 *18:52, 18:105	*52:28 *35:02:02, 35:20:02 C*06:60, C*07:427
31	135 bp	1070 bp	*18:49, 18:79, 18:108	*35:27, 35:56, 35:185, 35:203, 37:05, 78:06, 78:09
32⁹	-	-	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*18 SSP typings.

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Lot-specific information

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 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.

⁴Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B*18 alleles will be amplified by primer mixes 1 to 5, 7, 8, 10 to 18, 20 to 22, 24 to 31. In addition, a few HLA-A and HLA-C alleles will be amplified by primer mixes 2, 8, 9, 11, 13, 15, 16, 18, 21, 22, 26, 27 and 30.

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

⁶Primer mixes 15, 17, 18, 20 and 30 may have tendencies of unspecific amplifications.

⁷Primer mixes 10 and 26 have a tendency to giving rise to primer oligomer formation.

⁸Primer mix 29 may give rise to a lower yield of HLA-specific PCR product than the other B*18 primer mixes.

⁹Primer 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.

‘w’, might be weakly amplified.

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Lot No.: **5D1**

Lot-specific information

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	170	260	260	100	115	130	90	180	125	85	125	80
					145	245	150		255	175	610	140
							230				635	
Length of int. pos. control ¹	800	800	800	800	1070	1070	1070	1070	1070	800	1070	1070
5'-primer(s) ²	161 5'-Cgg 3'	363 5'-Agg 3'	363 5'-AAT 3'	234 5'-CAA 3'	130 5'-AgT 3'	363 5'-Agg 3'	161 5'-Cgg 3'	412 5'-ATg 3'	368 5'-gTT 3'	523 5'-CCA 3'	234 5'-CAA 3'	161 5'-Cgg 3'
		363 5'-Agg 3'			141 5'-ATT 3'		363 5'-Agg 3'	3 rd I 5'-Agg 3'	368 5'-gTg 3'	610 5'-TgT 3'		363 5'-Agg 3'
					161 5'-CgA 3'				499 5'-TCg 3'			
3'-primer(s) ³	292 5'-gTA 3'	583 5'-gTg 3'	583 5'-gTg 3'	292 5'-gTC 3'	234 5'-TCT 3'	453 5'-TCC 3'	272 5'-TgA 3'	559 5'-CTC 3'	583 5'-gTg 3'	3 rd I 5'-TAT 3'	317 5'-ggA 3'	197 5'-gAg 3'
	292 5'-gTA 3'					568 5'-CTT 3'	414 5'-TgC 3'	704 5'-CCA 3'			559 5'-CAg 3'	257 5'-TCg 3'
	294 5'-CgA 3'						553 5'-CTA 3'				583 5'-gTA 3'	259 5'-CTC 3'
												414 5'-TgC 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. PCR product	85	110	100	95	270	85	105	290	215	130	145	205
	265	170		360		175	160			150	160	
		260				200	405			270	515	
Length of int. pos. control ¹	1070	1070	1070	1070	1070	1070	1070	1070	1070	800	1070	1070
5'-primer(s) ²	361 5'-AgT 3'	106 5'-CCA 3'	523 5'-CCg 3'	41 5'-CTA 3'	355 5'-CCA 3'	97 5'-TCC 3'	234 5'-CAA 3'	409 5'-ggT 3'	409 5'-ggC 3'	161 5'-Cgg 3'	234 5'-CAA 3'	419 5'-gTA 3'
	539 5'-gCg 3'	167 5'-gCT 3'	527 5'-TgA 3'	486 5'-ACC 3'		369 5'-TAC 3'	3 rd I 5'-Agg 3'		409 5'-ggC 3'	355 5'-TCA 3'	3 rd I 5'-Agg 3'	
	539 5'-gTg 3'	363 5'-AgC 3'								499 5'-TCg 3'		
3'-primer(s) ³	583 5'-gTg 3'	234 5'-TCT 3'	583 5'-gTg 3'	234 5'-TCT 3'	583 5'-gTg 3'	142 5'-TgC 3'	299 5'-TCA 3'	3 rd I 5'-TAT 3'	583 5'-gTg 3'	257 5'-TCg 3'	340 5'-ggT 3'	583 5'-gTg 3'
		583 5'-gTg 3'		538 5'-CCA 3'		505 5'-gCT 3'	353 5'-ggC 3'			272 5'-TgC 3'	463 5'-gCg 3'	
						527 5'-CCT 3'	685 5'-ggg 3'			583 5'-gTg 3'	685 5'-ggg 3'	
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

101.519-12 – including *Taq* pol., IFU-01
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Lot No.: **5D1**

Lot-specific information

Well No.	25	26	27	28	29	30	31
Length of spec.	75	90	80	100	65	100	135
PCR product	190	115	135	175	120	145	
		180	175	200	255	420	
						495	
Length of int.	1070	1070	800	800	1070	800	1070
pos. control ¹							
5'-primer(s) ²	161	97	161	161	103	160	206
	5'-Cgg 3'	5'-TCT 3'	5'-Cgg 3'	5'-Cgg 3'	5'-CCT 3'	5'-ACC 3'	5'-gAC 3'
	463	160	419	369	368	234	
	5'-TgA 3'	5'-ACC 3'	5'-gTC 3'	5'-TAC 3'	5'-gTC 3'	5'-CAA 3'	
		186			560	478	
		5'-AgA 3'			5'-CgA 3'	5'-CgA 3'	
						523	
						5'-CCg 3'	
3'-primer(s) ³	193	234	197	218	181	368	302
	5'-CgT 3'	5'-TCT 3'	5'-gAg 3'	5'-gCT 3'	5'-gTA 3'	5'-CAT 3'	5'-ggT 3'
	301		204	322	583	583	
	5'-gTC 3'		5'-TCT 3'	5'-gTg 3'	5'-gTg 3'	5'-gTg 3'	
	322		294	502			
	5'-gTg 3'		5'-CgT 3'	5'-CTT 3'			
	605		506	506			
	5'-gCT 3'		5'-TgA 3'	5'-TgA 3'			
			518				
			5'-CCT 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 No.: **5D1**

Lot-specific information

CELL LINE VALIDATION SHEET																			
HLA-B*18 SSP subtyping kit ²																			
					Well														
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
					201663101	201443902	201443903	201443904	201443905	201663106	201443907	201443908	201443909	201663110	201443911	201443912	201443913	201663114	201443915
					Prod. No.:	201663101	201443902	201443903	201443904	201443905	201663106	201443907	201443908	201443909	201663110	201443911	201443912	201443913	201663114
IHCW 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	9004	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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

101.519-12 – including *Taq* pol., IFU-01
101.519-12u – without *Taq* pol., IFU-02

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Lot No.: **5D1**

Lot-specific information

CELL LINE VALIDATION SHEET																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
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Lot No.: 5D1

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 5 to 7, 9 to 12, 14, 16, 17, 19, 22, 23, 25 to 28 and 30 were available. The specificities of the primers in primer solutions 5, 7, 9, 11, 12, 14, 16, 17, 19, 22, 23, 25 to 28 and 30 were tested by separately adding additional 5'-primers, respectively 3'-primers.

In primer solution 6 only the 5'-primer was possible to test, the 3'-primer was not possible to test. In primer solution 10 only the 3'-primer was possible to test, the 5'-primer was not possible to test.

In primer solutions 5, 9, 15, 16, 21, 22, 26 and 30 one, two or three 5'-primers were not possible to test, and in primer solutions 1, 7, 8, 12, 18, 19, 22, 23, 25 and 27 to 29 one, two or three 3'-primers were not possible to test. Additional primers in primer solutions 8, 13, 18 and 29 were tested by separately adding one 5'-primer or one or two 3'-primers.

101.519-12 – including *Taq* pol., IFU-01
101.519-12u – without *Taq* pol., IFU-02

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Lot No.: 5D1

Lot-specific information

101.519-12 – including *Taq* pol., IFU-01
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101.519-12u – without *Taq* pol., IFU-02

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Lot No.: **5D1**

Lot-specific information

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