

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

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

Lot No.: **4H4**

Lot-specific information

Olerup SSP[®] HLA-B*48

Product number:	101.546-06 – including <i>Taq</i> polymerase 101.546-06u – without <i>Taq</i> polymerase
Lot number:	4H4
Expiry date:	2021-07-01
Number of tests:	6
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. 4H4.

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*48 LOT (8E2).

The HLA-B*48 kit is updated for new alleles to enable separation of:

- Null and Alternatively expressed alleles
- The product documentation has been updated for new alleles of IMGT 3.34.0

The format of the Worksheet has been changed.

The HLA-B*48 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP[®]* HLA-B*48 lot was made (Lot No. 8E2).

The HLA-B*48 primer set is unchanged compared to the previous *Olerup SSP[®]* HLA-B*48 (Lot No. 8E2).

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Lot No.: **4H4**

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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.

101.546-06 – including *Taq* pol., IFU-01

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 “Instructions for Use” (IFU)

Lot No.: **4H4**

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PRODUCT DESCRIPTION

HLA-B*48 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*48:01 to B*48:47 alleles.

PLATE LAYOUT

Each HLA-B*48 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 PCR plate is marked with ‘HLA-B*48’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘4H4’.

Wells 1 to 19 – HLA-B*48 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

Due to the sharing of sequence motifs between HLA-B alleles, non-HLA-B*48 alleles will be amplified by some primer mixes. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*48 alleles, i.e. **B*48:01 to B*48:47**, recognized by the HLA Nomenclature Committee in October 2018^{1,2} will be amplified by the primers in the HLA-B*48 SSP kit³.

The HLA-B*48 kit enables separation of the confirmed HLA-B*48 alleles as listed in the IMGT/HLA database 3.26.0. 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*48 alleles is listed below.

The HLA-B*48 kit also enables identification of null and alternatively expressed alleles.

101.546-06 – including *Taq* pol., IFU-01

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Lot No.: **4H4**

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The following HLA-B*48 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix
B*48:09, 48:11	14
B*48:18, 48:29	15
B*48:19, 48:27	9

¹HLA-B alleles listed on the IMGT/HLA web page 2018-October-18, release 3.34.0, 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>.

³The B*48 primer set cannot separate the B*48:10 and the B*39:13:02 and 42:11 alleles. These alleles can be distinguished by the HLA-B low resolution and/or HLA-B*39 and HLA-B*42 kits.

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101.546-06u – without *Taq* pol., IFU-02

Lot No.: **4H4**

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ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
B*48:01:01	Confirmed	B*48:12	Confirmed	B*48:32	Unconfirmed
B*48:01:02	Unconfirmed	B*48:13	Confirmed	B*48:33	Unconfirmed
B*48:01:03	Unconfirmed	B*48:14	Unconfirmed	B*48:34	Confirmed
B*48:01:04	Unconfirmed	B*48:15	Unconfirmed	B*48:35	Unconfirmed
B*48:01:05	Unconfirmed	B*48:16	Unconfirmed	B*48:36	Unconfirmed
B*48:01:06	Unconfirmed	B*48:17	Confirmed	B*48:37	Unconfirmed
B*48:02:01	Confirmed	B*48:18	Confirmed	B*48:38	Unconfirmed
B*48:02:02	Unconfirmed	B*48:19	Unconfirmed	B*48:39	Unconfirmed
B*48:02:03	Confirmed	B*48:20	Unconfirmed	B*48:40	Unconfirmed
B*48:03:01	Confirmed	B*48:21	Unconfirmed	B*48:41	Unconfirmed
B*48:03:02	Confirmed	B*48:22	Unconfirmed		
B*48:04:01	Confirmed	B*48:23	Unconfirmed		
B*48:04:02	Unconfirmed	B*48:24	Confirmed		
B*48:05	Confirmed	B*48:25	Unconfirmed		
B*48:06	Unconfirmed	B*48:26	Unconfirmed		
B*48:07	Confirmed	B*48:27	Unconfirmed		
B*48:08	Unconfirmed	B*48:28	Unconfirmed		
B*48:09	Unconfirmed	B*48:29	Unconfirmed		
B*48:10	Unconfirmed	B*48:30	Unconfirmed		
B*48:11	Unconfirmed	B*48:31	Unconfirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2016-October-14, release 3.26.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-B*48 homo- and heterozygotes is available upon request.

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Lot No.: **4H4**

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Lot-specific information
SPECIFICITY TABLE

HLA-B*48 SSP subtyping

Specificities and sizes of the PCR products of the 19+1 primer mixes used for
 HLA-B*48 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*48 alleles ³	Other amplified HLA-B alleles
1	385 bp	800 bp	*48:01:01:01-48:05, 48:07-48:15, 48:17- 48:38, 48:40-48:47	*08:17, 08:38:01-08:38:02, 08:54:01-08:54:02, 08:101, 15:68, 38:03-38:04, 38:25, 39:02:01- 39:02:03, 39:08, 39:13:01-39:13:02, 39:22-39:23, 39:49, 39:101, 39:105, 39:110, 40:01:01-40:01:58, 40:06:16 [?] , 40:07, 40:09 [?] , 40:14:01 [?] -40:14:02 [?] , 40:14:03, 40:15 [?] , 40:16, 40:18 [?] -40:19 [?] , 40:21 [?] - 40:22N [?] , 40:23, 40:24 [?] , 40:27:02 [?] -40:29 [?] , 40:30- 40:32, 40:33 [?] -40:36 [?] , 40:38 [?] , 40:42-40:43, 40:44 [?] - 40:47 [?] , 40:48, 40:49 [?] , 40:51-40:52, 40:53 [?] , 40:54, 40:55 [?] -40:58 [?] , 40:59-40:60, 40:61 [?] -40:63 [?] , 40:65 [?] , 40:66, 40:67 [?] , 40:71 [?] , 40:72:01-40:73, 40:74 [?] - 40:76 [?] , 40:77, 40:78 [?] , 40:79, 40:80 [?] -40:88 [?] , 40:91 [?] - 40:93 [?] , 40:95 [?] -40:96 [?] , 40:98 [?] , 40:100 [?] -40:105 [?] , 40:107 [?] -40:112 [?] , 40:113-40:114:01, 40:114:02 [?] - 40:116 [?] , 40:117, 40:118N [?] -40:121 [?] , 40:123 [?] , 40:124:01, 40:124:02 [?] -40:130:01 [?] , 40:130:02, 40:131 [?] -40:137 [?] , 40:138-40:140, 40:141 [?] , 40:145 [?] , 40:146-40:147, 40:148 [?] -40:149 [?] , 40:150, 40:151 [?] , 40:152-40:153, 40:154 [?] , 40:155:01N-40:155:02N, 40:156 [?] -40:165 [?] , 40:167 [?] -40:169 [?] , 40:170-40:172, 40:173 [?] -40:182 [?] , 40:183, 40:184 [?] -40:185 [?] , 40:186:01, 40:186:02 [?] -40:213 [?] , 40:215 [?] -40:228 [?] , 40:230 [?] -40:231 [?] , 40:233 [?] -40:236 [?] , 40:237, 40:238 [?] - 40:243 [?] , 40:245, 40:246 [?] -40:248 [?] , 40:249-40:253, 40:256N [?] -40:257 [?] , 40:258-40:265N, 40:272-40:273, 40:274 [?] -40:276 [?] , 40:277-40:278, 40:279 [?] -40:283 [?] , 40:285-40:286N, 40:287 [?] -40:295 [?] , 40:297 [?] -40:300 [?] , 40:301, 40:306 [?] -40:308 [?] , 40:310 [?] -40:312 [?] , 40:315 [?] , 40:316-40:317, 40:318 [?] -40:319 [?] , 40:321, 40:322 [?] , 40:323, 40:324 [?] -40:330 [?] , 40:332, 40:333 [?] , 40:335 [?] - 40:344 [?] , 40:346, 40:348 [?] -40:352 [?] , 40:354 [?] , 40:355, 40:358 [?] -40:361N [?] , 40:363 [?] -40:366 [?] , 40:367-40:368, 40:370, 40:372N, 40:374 [?] , 40:376 [?] , 40:377, 40:380- 40:383, 40:385-40:386, 40:388 [?] -40:389 [?] , 40:391- 40:395, 40:396 [?] , 40:397-40:398, 40:400 [?] , 41:01:01:01-41:56, 42:11, 44:15:01:02, 44:18, 45:01:01-45:05, 45:07-45:21, 45:23, 49:01:01:01- 49:14, 49:16-49:25, 49:27-49:59, 50:01:01:01- 50:02, 50:04-50:14, 50:16-50:20, 50:31-50:59
2	145 bp	800 bp	*48:01:01:01- 48:01:07, 48:03:01- 48:03:02, 48:06- 48:07, 48:09, 48:11, 48:15-48:23, 48:26- 48:47	*07:174, 07:202, 07:222, 07:279, 27:36, 27:125, 38:63, 40:01:01-40:01:29, 40:01:31-40:01:45, 40:01:47-40:01:58, 40:07, 40:10:01:01-40:10:02, 40:12, 40:21-40:23, 40:25, 40:30-40:31, 40:33- 40:34, 40:36, 40:38, 40:42-40:43, 40:45-40:49, 40:52, 40:54-40:55, 40:59-40:61, 40:63,

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

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

Lot No.: 4H4

Lot-specific information

				40:66-40:67, 40:69, 40:73-40:74, 40:76, 40:79-40:81, 40:84, 40:87:01-40:88, 40:100-40:102, 40:106, 40:112-40:114:02, 40:116-40:118N, 40:123-40:126, 40:128, 40:132, 40:134-40:135, 40:137-40:141, 40:146-40:147, 40:149-40:156, 40:160:01-40:160:02, 40:166, 40:168, 40:170-40:172, 40:175, 40:179, 40:182-40:183, 40:185-40:188, 40:191-40:199, 40:204, 40:207-40:208, 40:210, 40:212-40:213, 40:215-40:218, 40:221-40:223, 40:227-40:228, 40:231, 40:233-40:236, 40:239-40:242, 40:245, 40:247, 40:249-40:253, 40:257-40:265N, 40:272-40:273, 40:277-40:282, 40:285-40:286N, 40:288, 40:298:01-40:301, 40:308, 40:310, 40:315-40:316, 40:319, 40:321, 40:323-40:325, 40:327-40:329, 40:332-40:333, 40:338N-40:339, 40:344, 40:346, 40:352-40:353, 40:355, 40:357-40:358, 40:360, 40:365, 40:367-40:368, 40:370, 40:372N, 40:377, 40:380-40:383, 40:385-40:386, 40:391-40:397, 40:400, 44:31, 46:06, 81:01:01-81:08
3	175 bp	800 bp	*48:01:01:01-48:01:07, 48:04:01-48:04:02, 48:06-48:07, 48:09, 48:11, 48:15-48:16, 48:18-48:20, 48:22, 48:24, 48:27-48:39, 48:41-48:47	*07:174, 07:202, 07:222, 07:279, 27:24, 40:31, 40:45, 40:80, 81:01:01-81:08
4	150 bp	800 bp	*48:02:01-48:02:03, 48:25	*07:235, 08:49, 13:01:01:01-13:01:14, 13:06-13:07N, 13:12:01-13:13:02, 13:17, 13:20, 13:22:01-13:23, 13:25-13:26:02, 13:28-13:29, 13:36, 13:39, 13:43, 13:50-13:52, 13:57, 13:60-13:61, 13:63N, 13:73, 13:76N-13:80, 13:83, 13:86-13:87, 13:90, 13:92, 13:95, 13:106-13:107, 13:109-13:110, 13:122, 14:10, 15:02:01:01-15:02:12, 15:13:01-15:13:02, 15:20-15:21:01:02, 15:25:01-15:25:03, 15:36, 15:44, 15:62, 15:77, 15:80, 15:85, 15:88-15:89, 15:106, 15:112, 15:121, 15:139, 15:144, 15:154, 15:165, 15:170, 15:194, 15:204, 15:213-15:214, 15:223, 15:240, 15:250, 15:265, 15:271, 15:283, 15:289, 15:291, 15:297, 15:301-15:302N, 15:308, 15:319, 15:325, 15:328, 15:330, 15:341, 15:345, 15:357-15:358, 15:366, 15:374, 15:378, 15:393-15:394, 15:399, 15:402, 15:404, 15:407, 15:418, 15:420, 15:425, 15:437-15:438, 15:454N, 15:458, 15:460, 15:463N, 15:468, 18:22, 18:69, 18:105, 18:149-18:150, 27:19, 27:30, 27:127, 27:150, 35:01:01:01-35:04:03, 35:06-35:08:10, 35:10-35:17:02, 35:19-35:21, 35:23-35:30, 35:33-35:36, 35:38-35:42:02, 35:45-35:50, 35:52, 35:54-35:57, 35:59:01-35:59:02, 35:61:01-35:63, 35:65Q, 35:69-35:71, 35:74, 35:76-35:78, 35:80-35:85, 35:90-35:96, 35:98, 35:100-35:101:02, 35:103-35:113, 35:115-35:116, 35:120-35:126, 35:128-35:134N, 35:136-35:150:02, 35:152-35:173:02N, 35:175-35:184, 35:186-35:198, 35:200-35:204, 35:206-35:229, 35:231, 35:233, 35:236-35:250,

101.546-06 – including Taq pol., IFU-01

101.546-06u – without Taq pol., IFU-02

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

Lot No.: 4H4

Lot-specific information

				35:252-35:259, 35:261-35:264, 35:266-35:272, 35:274-35:275, 35:277-35:291, 35:293-35:320, 35:322-35:330, 35:332-35:366, 35:370-35:371, 35:373-35:388, 35:390N-35:396, 37:01:01:01-37:01:07, 37:01:09, 37:01:11-37:01:14, 37:03N-37:06:02, 37:08, 37:10-37:11, 37:13-37:43, 37:45-37:50, 37:52-37:76, 38:20:01-38:20:02, 39:42, 40:28, 44:02:01:01-44:02:33, 44:02:35-44:14, 44:16-44:17, 44:19N, 44:21-44:30, 44:32-44:40, 44:42-44:46, 44:48-44:52N, 44:55-44:64:02, 44:66-44:98, 44:101-44:105, 44:107-44:134, 44:136-44:137, 44:139-44:157, 44:159-44:165, 44:167-44:181, 44:183, 44:185-44:191, 44:194-44:196, 44:198N-44:212, 44:214-44:229, 44:231-44:235, 44:237N-44:245, 44:247-44:297, 46:33, 46:67, 50:17, 51:04:01-51:04:02, 51:42, 51:46, 51:56:01-51:56:03, 51:139, 51:227, 53:01:01:01-53:13, 53:15-53:40, 53:42-53:43, 53:45-53:46, 53:48N-53:52, 55:14, 56:09, 56:11-56:12, 56:60, 57:01:01:01-57:01:04, 57:01:06-57:10, 57:12, 57:14:01-57:20, 57:22-57:30, 57:32-57:46, 57:48-57:73, 57:75-57:81, 57:84-57:110, 58:01:01:01-58:01:02, 58:01:04-58:01:15, 58:01:17-58:01:29, 58:04-58:05, 58:09-58:15, 58:17N, 58:19, 58:21-58:24, 58:28:01-58:29, 58:31N-58:37, 58:39N-58:42, 58:44-58:45:02, 58:47-58:59:02, 58:62-58:97, 83:01, C*03:102, C*03:263:01-03:263:02, C*15:126
5	230 bp 285 bp	1070 bp	*48:26 *48:03:01-48:03:02, 48:17, 48:23, 48:40	*07:197, 15:230, 27:36, 40:124:01-40:124:02 *07:07, 07:12, 07:14, 07:18:01-07:18:02, 07:77, 07:137, 07:162-07:163, 07:193, 07:214, 07:248, 07:255, 15:138, 15:230, 15:241, 15:297, 18:35, 18:110, 27:125, 38:63, 40:01:01-40:01:17, 40:01:19-40:01:58, 40:07, 40:10:01:01-40:10:02, 40:12, 40:14:01-40:14:03, 40:21-40:23, 40:25, 40:33, 40:36, 40:38, 40:42-40:43, 40:46-40:49, 40:52, 40:54-40:55, 40:59, 40:60-40:63, 40:65-40:67, 40:69, 40:72:01-40:74, 40:76-40:77, 40:79, 40:81, 40:84, 40:87:01-40:88, 40:100-40:102, 40:106, 40:108, 40:112-40:114:02, 40:116-40:118N, 40:121, 40:123-40:126, 40:128, 40:132, 40:134-40:136, 40:137, 40:138-40:141, 40:146-40:147, 40:149-40:156, 40:158, 40:160:01-40:160:02, 40:166, 40:168, 40:170-40:172, 40:175, 40:179, 40:182-40:183, 40:185-40:188, 40:191-40:199, 40:204, 40:207-40:208, 40:210, 40:212-40:213, 40:215-40:218, 40:221-40:223, 40:227-40:228, 40:231, 40:234-40:242, 40:245, 40:247, 40:249-40:253, 40:257-40:265N, 40:272-40:273, 40:277-40:282, 40:285-40:286N, 40:288, 40:299-40:301, 40:308, 40:310, 40:312, 40:315, 40:319, 40:321, 40:323-40:329, 40:332-40:333, 40:338N-40:339, 40:344, 40:346, 40:352-40:353, 40:355, 40:357-40:358, 40:360, 40:365, 40:367-40:368, 40:370, 40:372N, 40:377, 40:380-40:383, 40:385-40:386, 40:391-40:397, 40:400, 41:08, 44:31,

101.546-06 – including Taq pol., IFU-01

101.546-06u – without Taq pol., IFU-02

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

Lot No.: 4H4

Lot-specific information

				44:150, 46:06, C*02:23, C*03:260, C*04:77, C*07:38:01-07:38:02
6⁴	90 bp 170 bp 285 bp	1070 bp	*48:20 *48:05 *48:21, 48:26	*07:197, 27:36
7	385 bp	1070 bp	*48:06	*07:02:01:01-07:12, 07:14-07:53, 07:55-07:64, 07:66-07:122, 07:124-07:131, 07:133-07:180, 07:182N, 07:183 ^w , 07:184-07:218, 07:220-07:258, 07:260-07:270, 07:272N-07:289, 07:291-07:335, 08:01:01:01-08:05, 08:07-08:09, 08:11-08:16, 08:18-08:37, 08:39-08:53:02, 08:55-08:100, 08:102-08:146, 08:149-08:208, 14:01:01:01-14:03, 14:05-14:48, 14:50-14:56, 14:57 ^w , 14:58-14:66, 38:01:01:01-38:02:08, 38:05-38:24, 38:26-38:55Q, 38:57-38:79, 39:01:01:01-39:01:01:09, 39:01:03:01-39:01:24, 39:03:01:01-39:07, 39:09:01:01-39:10:01, 39:11-39:12:01:02, 39:14:01:01-39:20, 39:24:01-39:48, 39:50-39:77, 39:79-39:100, 39:102-39:104, 39:106-39:109, 39:111-39:141, 40:25 [?] , 40:68 [?] , 40:106 [?] , 40:166 [?] , 40:232 [?] , 40:313 [?] , 42:01:01-42:02:01:02, 42:04-42:10, 42:12-42:25, 45:22, 49:26, 50:15, 67:01:01-67:01:03, 67:03-67:07, 73:01 ^w -73:02 ^w , 81:02
8^{4,5}	85 bp 225 bp	1070 bp	*48:16 *48:07, 48:17	*07:78, 07:115, 07:214, 07:327, 38:63, 40:36, 40:48, 40:77, 40:280, 40:368, 40:377, C*04:77
9^{4,6}	75 bp 215 bp	800 bp	*48:27 *48:05, 48:08, 48:19, 48:24	*07:74, 08:77 *07:02:01:01-07:02:25, 07:02:27-07:02:38, 07:02:40-07:03, 07:05:01:01-07:06:03, 07:08:01-07:11, 07:13, 07:15-07:17, 07:20-07:24, 07:27-07:33:03, 07:35-07:42, 07:44N, 07:46-07:47, 07:49N-07:51, 07:53-07:65, 07:67N-07:76, 07:79, 07:81-07:83, 07:85:01-07:130, 07:132-07:134, 07:136:01-07:136:02, 07:138-07:145, 07:148-07:161N, 07:165-07:177, 07:179-07:192, 07:194-07:195, 07:199-07:208, 07:210-07:213, 07:215-07:222, 07:224, 07:228:01-07:234, 07:236-07:238, 07:240-07:247, 07:249-07:254, 07:256-07:335, 08:20:01-08:20:02, 08:40, 08:70, 08:79, 08:156, 15:07:01:01-15:07:03, 15:45, 15:68, 15:126, 15:207, 15:324, 15:331, 15:405, 15:431, 15:450, 35:66, 35:232, 37:07, 40:15-40:16, 40:32, 40:98, 46:12, C*02:60
10⁵	290 bp	800 bp	*48:04:01-48:04:02, 48:13, 48:24	*07:33:01, 07:53, 07:60, 07:100, 07:139:01, 08:116, 08:134, 08:175, 13:71, 13:108, 14:05, 14:13, 14:53, 15:07:01:01-15:07:03, 15:45, 15:55, 15:68, 15:126, 15:197:02, 15:207, 15:324, 15:329, 15:331, 15:360, 15:383, 15:405, 15:421, 15:431, 15:450, 18:14, 18:22, 27:07:01-27:07:06, 27:11, 27:24, 27:32-27:34, 27:43, 27:138, 27:180, 35:05:01:01-35:05:03, 35:16-35:17:02, 35:22, 35:30-35:31, 35:51, 35:58, 35:72, 35:89, 35:97, 35:113-35:114, 35:125, 35:164, 35:199, 35:232, 35:242, 35:318, 35:346, 35:368, 35:389, 37:09, 38:19, 39:03:01:01-39:03:01:02, 39:14:01:01-39:14:01:02, 39:24:01-39:24:02, 39:29, 39:37, 39:76, 39:120,

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

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 “Instructions for Use” (IFU)

Lot No.: **4H4**

Lot-specific information

				40:02:01:01-40:03:01:02, 40:05:01:01-40:05:01:02, 40:08-40:09, 40:13, 40:18-40:19, 40:24, 40:27:01-40:27:02, 40:29, 40:35:01-40:35:02, 40:37, 40:39-40:40, 40:50, 40:56-40:58, 40:71, 40:78, 40:82, 40:85, 40:89-40:91, 40:94, 40:97, 40:104-40:105, 40:107, 40:111, 40:115, 40:119, 40:122, 40:133Q, 40:142N-40:145, 40:157, 40:164, 40:169, 40:173-40:174, 40:176, 40:180-40:181, 40:189, 40:200-40:203, 40:205-40:206, 40:209, 40:211, 40:214, 40:219-40:220, 40:224, 40:226, 40:229, 40:232, 40:246, 40:248, 40:254-40:255, 40:266-40:267, 40:271, 40:274, 40:276, 40:283-40:284, 40:287, 40:289-40:293, 40:296-40:297, 40:302-40:305, 40:309, 40:314, 40:320, 40:322, 40:331, 40:334-40:335, 40:337N, 40:345N, 40:347, 40:356, 40:359, 40:369, 40:371, 40:375, 40:378, 40:384, 40:390, 40:399N, 41:15, 42:08, 42:14, 44:54, 44:106, 44:110, 44:135, 44:158, 44:184, 44:211, 44:213, 46:12, 51:64, 51:81, 51:148, 53:14, 55:04, 55:49, 55:51, 55:86, 58:18, 58:27, C*02:60, C*03:05, C*03:25, C*03:27, C*03:143, C*03:167, C*03:296:01-03:296:02, C*03:335, C*03:346, C*03:386, C*03:407, C*08:08:01-08:08:02, C*15:140
11 ⁶	150 bp	800 bp	*48:01:01:01-48:05, 48:07-48:27, 48:29-48:30, 48:32-48:47	*07:27, 07:50, 13:01:01:01-13:04, 13:06-13:23, 13:25-13:29, 13:31-13:53, 13:55-13:74, 13:76N-13:84, 13:86-13:100, 13:102-13:114, 13:116N-13:123Q, 15:01:01:01-15:01:03, 15:01:06-15:01:08, 15:01:10-15:02:12, 15:03:03, 15:04:01:01-15:07:03, 15:12:01-15:15, 15:19-15:20, 15:24:01-15:28, 15:30:01:01-15:36, 15:38:01-15:40:02, 15:42-15:43, 15:45-15:46, 15:48, 15:50, 15:55, 15:57-15:58, 15:60, 15:63, 15:65, 15:68, 15:70-15:71, 15:73, 15:75, 15:77-15:79N, 15:81-15:89, 15:92, 15:94N, 15:96, 15:102, 15:104-15:107, 15:109-15:113, 15:116-15:118, 15:120-15:122, 15:125-15:126, 15:128-15:129, 15:135-15:138, 15:140-15:142, 15:144-15:147, 15:150, 15:154-15:155, 15:157, 15:159-15:160, 15:163-15:167, 15:169-15:172, 15:174-15:175, 15:178-15:179:02, 15:181N-15:185, 15:187-15:188, 15:190N, 15:192-15:195, 15:199, 15:201-15:207, 15:211, 15:213-15:214, 15:217-15:219, 15:223-15:225, 15:227-15:228, 15:231-15:234, 15:237, 15:239-15:241, 15:244-15:251:02, 15:256-15:262N, 15:264-15:265, 15:267, 15:269-15:272N, 15:276-15:280, 15:283, 15:285-15:289, 15:291, 15:295-15:302N, 15:308-15:310, 15:315-15:322, 15:324-15:328, 15:330-15:334, 15:339-15:346, 15:348-15:349:02, 15:352-15:353, 15:355, 15:357-15:360, 15:363:01-15:366, 15:368, 15:371-15:375N, 15:377Q-15:378, 15:381, 15:383-15:387, 15:390-15:393, 15:395, 15:398-15:402, 15:405-15:407, 15:409-15:410, 15:412-15:413, 15:415-15:419, 15:421-15:422, 15:425, 15:428-15:432, 15:437-15:438, 15:440-15:443, 15:449-15:450, 15:452-15:454N, 15:456-15:461, 15:463N-15:467, 15:469-15:474, 15:476, 18:72:01,

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

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

Lot No.: **4H4**

Lot-specific information

				35:20:01-35:20:02, 35:28, 35:72, 35:95, 35:226, 35:296, 35:361, 37:01:01:01-37:01:06, 37:01:08-37:20, 37:22-37:25, 37:27-37:56, 37:58-37:59, 37:61-37:66, 37:68-37:76, 39:13:02, 40:02:04, 40:10:02, 40:21, 40:83, 40:137, 40:185, 40:209, 40:248, 40:362, 40:364-40:365, 42:11, 44:60, 44:83-44:84:01, 44:194, 44:205:01-44:205:02, 44:225, 44:269, 49:03, 51:07:01-51:07:02, 51:214, 51:222, 51:251, 52:01:01:01-52:01:12, 52:01:14-52:05, 52:07-52:21:02, 52:24-52:69, 52:71-52:82, 53:09, 53:17:01-53:17:02, 53:28, 53:38, 53:40, 78:05-78:06
12⁴	110 bp	1070 bp	*48:22, 48:30	*07:143, 13:23, 13:55, 15:71, 15:175, 15:204, 15:225, 15:344, 15:381, 18:01:01:01-18:01:12, 18:01:14-18:03:02, 18:05:01:01-18:15, 18:17N-18:24, 18:26-18:42, 18:44:01-18:72:01, 18:73-18:101, 18:103-18:106, 18:108-18:124, 18:126-18:127, 18:129-18:158, 27:75, 35:50, 35:84, 35:162, 35:231, 35:280, 35:323, 37:01:01:01-37:01:06, 37:01:08-37:54, 37:56-37:61, 37:63-37:76, 40:83, 40:185, 40:209, 40:248, 40:362, 40:364, 42:02:01:01-42:02:01:02, 42:09, 42:17-42:18, 46:14, 49:03, 51:116, 51:151, 52:51, 57:39, 57:73, 58:41, 73:01-73:02
	135 bp		*48:23	*15:116, 15:124, 18:88, 40:52, 40:59, 40:352, 46:06, 46:43, 57:59, 58:05, C*07:123, C*07:173, C*07:294
13⁷	135 bp	1070 bp	*48:05, 48:10, 48:12-48:13, 48:14 ^w	*07:05:01:01-07:06:03, 07:32, 07:34, 07:40, 07:53, 07:69, 07:78, 07:80, 07:97, 07:105, 07:112, 07:123, 07:137-07:138, 07:140, 07:176, 07:182N, 07:206-07:207, 07:209-07:210, 07:213-07:214, 07:225, 07:249, 07:258, 07:262, 07:264, 07:269-07:270, 07:278, 07:283, 07:287, 07:293, 07:304, 07:317, 07:324, 07:327, 07:332-07:333, 08:01:01:01-08:01:11, 08:01:13-08:01:22, 08:01:24-08:05, 08:08N-08:12:01, 08:12:03-08:13, 08:15-08:27, 08:29-08:36, 08:38:01-08:48, 08:50-08:63, 08:65-08:73, 08:75-08:82N, 08:84 ^w , 08:85-08:86N, 08:88, 08:90-08:106, 08:108-08:110, 08:112-08:155, 08:157-08:166, 08:167 ^w , 08:168-08:199, 08:201-08:208, 13:03, 13:22:01-13:22:02, 13:40 ^w , 13:45, 13:48, 13:62, 13:86, 14:13, 14:53, 15:09:01-15:09:02, 15:30:01:01-15:30:01:02, 15:42, 15:48, 15:58, 15:73, 15:83, 15:86, 15:150, 15:188, 15:224, 15:229, 15:243, 15:252, 15:303, 15:324, 15:329, 15:361, 15:435, 15:453, 15:460, 18:26, 18:61, 18:97, 27:07:01-27:07:06, 27:11, 27:20, 27:27, 27:33, 27:43, 27:130, 27:153, 27:164, 27:168, 27:180, 35:02:01:01-35:02:11, 35:04:01-35:04:03, 35:06, 35:09:01-35:09:03, 35:12:01-35:12:04, 35:18, 35:22, 35:31, 35:44, 35:59:01-35:60, 35:81, 35:83, 35:87-35:88, 35:95, 35:129N, 35:146, 35:149, 35:154, 35:157, 35:162, 35:172, 35:182-35:184, 35:201, 35:211, 35:220, 35:233, 35:251, 35:258, 35:266, 35:270, 35:273, 35:276, 35:285, 35:292, 35:309, 35:311, 35:316, 35:321, 35:323, 35:335, 35:339, 35:357, 35:361, 35:366-35:367,

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

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“Instructions for Use” (IFU)

Lot No.: **4H4**

Lot-specific information

35:372, 35:374, 35:377-35:379, 35:384, 35:387,
35:391, 35:394, 35:396^w, 37:12, 38:01:01:01-
38:01:01:02, 38:01:03-38:02:05, 38:02:07-38:21,
38:23-38:28, 38:30-38:37, 38:39-38:48, 38:49^w,
38:50-38:51, 38:53-38:62, 38:64-38:69, 38:71,
38:73-38:79, 39:01:01:01-39:01:01:09, 39:01:03:01-
39:01:18, 39:01:20-39:06:06, 39:08-39:14:01:02,
39:16, 39:18-39:20, 39:22, 39:24:01-39:24:02,
39:26-39:28, 39:30-39:42, 39:45-39:48, 39:50-
39:54, 39:56^w, 39:57-39:90, 39:92-39:95N, 39:97N,
39:98^w, 39:99-39:108, 39:109^w, 39:110-39:140,
40:02:01:01-40:02:09, 40:02:11-40:02:17, 40:02:19-
40:02:25, 40:04-40:06:09, 40:06:11-40:06:16,
40:08, 40:11:01-40:11:02, 40:13-40:16, 40:19,
40:26-40:29, 40:35:01-40:35:02, 40:37, 40:39-
40:40, 40:50, 40:53, 40:56-40:58, 40:64:01:01-
40:64:01:02, 40:68, 40:70:01-40:71, 40:72:01^w-
40:72:02^w, 40:75, 40:77^w, 40:78, 40:82-40:83,
40:86, 40:90, 40:93-40:97, 40:99, 40:103-40:105,
40:107, 40:109-40:111, 40:115, 40:119, 40:121-
40:122, 40:127, 40:129, 40:133Q, 40:136, 40:142N-
40:145, 40:148, 40:157-40:158, 40:161-40:162,
40:164-40:165, 40:167, 40:169, 40:173-40:174,
40:176-40:177, 40:181, 40:184, 40:189-40:190,
40:200-40:203, 40:205-40:206, 40:209, 40:211,
40:214, 40:219-40:220, 40:224-40:226, 40:229-
40:230, 40:232, 40:237, 40:243-40:244, 40:246,
40:248, 40:254-40:256N, 40:266, 40:268-40:271,
40:274-40:276, 40:283, 40:287, 40:289, 40:290^w,
40:291N-40:297, 40:302-40:304, 40:305^w, 40:306,
40:309, 40:311, 40:313-40:314, 40:317-40:318,
40:320, 40:322, 40:331, 40:334-40:335, 40:337N,
40:340, 40:342-40:343, 40:345N, 40:347-40:351,
40:354, 40:356, 40:359, 40:361N-40:364, 40:366,
40:369, 40:371^w, 40:373-40:376, 40:378, 40:384,
40:387-40:390, 40:399N, 41:01:01:01-41:03:02,
41:05-41:09, 41:11-41:17, 41:19-41:20, 41:22-
41:24, 41:25^w, 41:26-41:36, 41:38-41:48, 41:49^w,
41:50, 41:53^w, 41:54-41:56, 42:01:01-42:02:01:02,
42:04-42:08, 42:10-42:15, 42:17-42:19, 42:21-
42:25, 44:10, 44:14, 44:62, 44:77, 44:82, 44:166,
44:184, 44:213, 45:12, 45:14, 46:11, 46:18, 46:61,
46:74, 50:36, 51:01:01:01-51:01:18, 51:01:19^w,
51:01:20-51:01:30, 51:01:32-51:01:49, 51:01:51-
51:02:02, 51:02:04-51:16, 51:19-51:24:05, 51:26,
51:28-51:36, 51:38-51:41N, 51:43-51:44N, 51:46,
51:48-51:74, 51:75^w, 51:76-51:83, 51:86-51:89,
51:91, 51:95-51:121, 51:123-51:129, 51:131-
51:134, 51:136-51:145, 51:147-51:162, 51:164-
51:171, 51:173Q-51:176, 51:178N-51:194, 51:196,
51:198-51:205, 51:206^w, 51:207-51:215, 51:217-
51:219, 51:221-51:222, 51:224-51:226, 51:228-
51:251, 51:252^w, 51:253, 51:255, 52:01:01:01-
52:01:10, 52:01:12-52:01:19, 52:01:20^w, 52:01:21-
52:01:22, 52:01:23^w, 52:01:24-52:11, 52:13-
52:25:02, 52:27-52:56, 52:57^w, 52:58-52:62, 52:64-
52:74, 52:76-52:82, 53:19, 53:36, 53:50,

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

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

Lot No.: 4H4

Lot-specific information

				54:01:01-54:05N, 54:07-54:08N, 54:10, 54:12-54:13, 54:17-54:21, 54:23-54:26, 54:28-54:38, 55:01:01:01-55:05, 55:07-55:13, 55:15-55:22, 55:24-55:26, 55:28-55:40, 55:42-55:45, 55:47-55:49, 55:51-55:57, 55:59-55:67, 55:69-55:85, 55:86 ^w , 55:88-55:95, 56:01:01:01-56:02:01, 56:02:02 ^w , 56:04:01-56:08, 56:10, 56:12-56:17, 56:19N-56:30, 56:33-56:49, 56:51-56:59, 56:62, 57:02:01-57:03:04, 57:07, 57:09, 57:12, 57:17, 57:28N, 57:39, 57:42 ^w , 57:46, 57:57, 57:63, 57:66, 57:70, 57:80, 57:82, 57:84, 57:94, 57:96, 57:101, 57:107, 58:08:01-58:08:02, 58:20, 58:27-58:28:02, 58:65, 58:73, 59:01:01:01-59:10N, 67:01:01-67:07, 78:01:01:01-78:10, 82:01:01:01-82:03, A*26:01:19^w, C*01:90, C*01:116, C*02:68, C*02:125, C*03:317, C*06:21, C*14:92, C*15:24, C*15:91, C*15:128
14⁴	100 bp	1070 bp	*48:09, 48:14	*07:09:01-07:09:02, 07:11, 07:17, 07:237, 07:287, 08:28, 08:35, 08:37, 15:07:01:01-15:07:03, 15:55, 15:68, 15:126, 15:197:02, 15:207, 15:331, 15:360, 15:383, 15:405, 15:421, 15:431, 15:450, 18:14, 35:05:01:01-35:05:03, 35:51, 35:58, 35:66, 35:72, 35:89, 35:97, 35:114, 35:232, 35:368, 35:389, 40:03:01:01-40:03:01:02, 40:105, 40:267, 40:284, 41:24, 41:51, 42:09, 46:12, 53:14, 58:18, C*02:60, C*07:294, C*07:526:01, C*07:530, C*07:681
	250 bp		*48:11	
15⁵	140 bp	1070 bp	*48:12, 48:14, 48:29	*07:68:01-07:68:03, 07:100, 07:237, 07:262, 08:71, 13:108, 14:05, 14:13, 14:53, 15:07:01:01-15:07:03, 15:45, 15:55, 15:68, 15:126, 15:197:02, 15:207, 15:324, 15:329, 15:331, 15:360, 15:383, 15:405, 15:431, 15:450, 18:14, 27:32, 27:138, 27:180, 35:05:01:01-35:05:03, 35:22, 35:51, 35:58, 35:66, 35:72, 35:89, 35:97, 35:199, 35:232, 35:368, 35:389, 38:19, 39:03:01:01-39:03:01:02, 39:14:01:01-39:14:01:02, 39:24:01-39:24:02, 39:29, 39:37, 39:76, 39:120, 40:35:01-40:35:02, 40:71, 41:24, 42:10, 42:17, 44:54, 44:106, 44:135, 44:158, 44:184, 44:213, 46:12, 51:64, 53:14, 58:18
	435 bp		*48:18	*07:36, 07:38, 07:81, 07:180, 07:219, 08:03, 08:52, 08:78, 38:01:01:01-38:01:13, 38:05-38:07, 38:09-38:14, 38:16, 38:19-38:22, 38:24-38:28, 38:30-38:34N, 38:36-38:42, 38:51-38:61, 38:63, 38:65-38:71, 38:73, 38:77-38:78, 40:19 [?] , 40:109 [?] , 40:117, 40:292 [?] , 40:340 [?] , 40:394, 41:46, 44:18, 49:01:01:01-49:01:13, 49:03-49:14, 49:16-49:59
16	130 bp	1070 bp	*48:05, 48:08, 48:15, 48:28	*07:02:01:01-07:18:02, 07:20-07:24, 07:26-07:32, 07:34-07:39, 07:41-07:47, 07:49N-07:50, 07:52, 07:54-07:59, 07:61-07:99, 07:101-07:121, 07:123-07:138, 07:140-07:154, 07:156-07:163, 07:166-07:201N, 07:203-07:208, 07:210-07:219, 07:221-07:222, 07:224-07:225, 07:227-07:234, 07:236-07:260, 07:262-07:300, 07:302-07:331, 07:333-07:335, 08:79, 08:156, 15:138, 15:230, 15:241, 15:297, 18:12:01-18:12:02, 37:07, 40:15-40:16, 40:23, 40:32, 40:98, 40:158, 40:183, 40:198, 40:355, 44:150, C*02:23, C*03:260, C*04:77

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

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 “Instructions for Use” (IFU)

Lot No.: **4H4**

Lot-specific information

17⁴	75 bp	1070 bp	*48:34	*07:169
18⁴	60 bp	1070 bp	*48:06, 48:31	*07:207, 07:228:01-07:228:02, 08:23, 08:133, 15:08:01-15:08:02, 15:29, 15:56, 15:191, 18:07:01-18:07:02, 18:29, 18:79, 27:23, 35:01:01:01-35:03:11, 35:03:13-35:03:17, 35:03:19-35:19, 35:21-35:25, 35:27, 35:30-35:43:01, 35:43:03-35:62, 35:64:01-35:68:02, 35:70-35:71, 35:74-35:75, 35:77-35:81, 35:83-35:84, 35:86-35:94, 35:96-35:111, 35:113-35:134N, 35:137-35:152, 35:154-35:185, 35:187-35:197, 35:199-35:202, 35:204-35:211, 35:213-35:225, 35:227-35:228, 35:230-35:242, 35:244-35:248, 35:250-35:253, 35:255-35:293, 35:295, 35:297-35:304, 35:306-35:315, 35:318-35:354, 35:356-35:360, 35:362-35:396, 37:67, 40:07-40:08, 40:68, 40:106, 40:162, 40:220, 40:232, 51:185, 51:247, 53:05:01-53:05:02, 53:16, 53:33, 78:02:01-78:02:02, 78:04, 78:09-78:10, C*06:147
19	150 bp	1070 bp	*48:25	*07:09:01 ^w -07:09:02 ^w , 07:11 ^w , 07:162 ^w , 07:237 ^w , 07:255 ^w , 15:03:01:01-15:03:08, 15:16:01:01-15:16:03, 15:18:01:01-15:18:07, 15:23, 15:29, 15:39:01-15:40:02, 15:47:01 ^w -15:47:02 ^w , 15:49 ^w , 15:61-15:62, 15:64:01-15:64:02, 15:65 ^w , 15:67, 15:69, 15:72, 15:74, 15:80, 15:93, 15:95, 15:98, 15:103, 15:108, 15:115, 15:119, 15:127, 15:131-15:132, 15:134, 15:138, 15:153, 15:158, 15:161, 15:173, 15:176, 15:194-15:195, 15:197:01-15:198, 15:210, 15:220, 15:221 ^w , 15:223, 15:226N, 15:238, 15:242:01-15:242:02, 15:254-15:255, 15:263, 15:266, 15:268, 15:274, 15:281-15:282, 15:288, 15:290, 15:292-15:294N, 15:306-15:307, 15:310-15:311, 15:314, 15:335, 15:337, 15:351, 15:354, 15:362, 15:364, 15:369, 15:376, 15:379 ^w , 15:380N, 15:382, 15:388-15:389, 15:397, 15:402, 15:408, 15:414, 15:426, 15:433-15:434, 15:436, 15:444, 15:447-15:448, 15:451, 15:475, 18:15, 18:21 ^w , 18:30 ^w , 18:57:01-18:57:02, 18:101, 18:152, 35:11:01-35:11:02, 35:11:03 ^w , 35:21, 35:58, 35:99 ^w , 35:188, 35:232 ^w , 35:277, 35:337:01 ^w -35:337:02 ^w , 46:03 ^w , 46:08, 46:62 ^w , 49:04:01-49:04:02, 51:37, 51:45, 53:06, 53:08:01-53:08:02, 53:28, 55:14, 55:68, 56:32, 57:13 ^w , 57:22, 58:06, 58:19, 58:46, 58:91, C*01:102^w, C*03:175, C*03:199, C*03:245, C*08:113, C*15:09, C*15:19, C*15:27, C*15:61, C*15:107, C*15:126, C*15:143^w
20⁸	-	-	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*48 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.

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

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Lot No.: **4H4**

Lot-specific information

PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

³For several HLA Class I alleles 1st and/or 4th exon(s) and beyond, as well as intron nucleotide sequences, are not available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

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

⁵Primer mixes 8, 10 and 15 may have tendencies of unspecific amplifications.

⁶Primer mixes 9 and 11 has a tendency to giving rise to primer oligomer formation.

⁷Primer mix 13 might faintly amplify the B*13 alleles.

⁸Primer 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.

‘w’, might be weakly amplified.

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

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

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Lot No.: **4H4**

Lot-specific information

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	385	145	175	150	230	90	385	85	75	290	150	110
					285	170		225	215			135
						285						
Length of int. pos. control ¹	800	800	800	800	1070	1070	1070	1070	800	800	800	1070
5'-primer(s) ²	44	499	363	355	363	144	44	260	363	363	165	97
	5'-ggC ^{3'}	5'-TCT ^{3'}	5'-AgC ^{3'}	5'-TCA ^{3'}	5'-Agg ^{3'}	5'-TCC ^{3'}	5'-ggC ^{3'}	5'-ggT ^{3'}	5'-AgC ^{3'}	5'-AgC ^{3'}	5'-ACC ^{3'}	5'-TCC ^{3'}
					363	225		419		363		419
					5'-Agg ^{3'}	5'-TgC ^{3'}		5'-gTT ^{3'}		5'-AgC ^{3'}		5'-gTC ^{3'}
					418	363		419				
					5'-Agg ^{3'}	5'-AAT ^{3'}		5'-gTT ^{3'}				
3'-primer(s) ³	259	605	499	463	605	272	259	302	398	610	272	165
	5'-CTC ^{3'}	5'-gCT ^{3'}	5'-ggA ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	5'-Tgg ^{3'}	5'-gTT ^{3'}	5'-ggC ^{3'}	5'-ggC ^{3'}	5'-CTg ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}
						605	259	605	537			512
						5'-gCT ^{3'}	5'-gTT ^{3'}	5'-gCT ^{3'}	5'-Agg ^{3'}			5'-CCA ^{3'}
									539			
									5'-TCC ^{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. PCR product	135	100	140	130	75	60	150
		250	435				
Length of int. pos. control ¹	1070	1070	1070	1070	1070	1070	1070
5'-primer(s) ²	397	363	44	161	693	272	419
	5'-gCT ^{3'}	5'-AgC ^{3'}	5'-ggC ^{3'}	5'-Cgg ^{3'}	5'-ggT ^{3'}	5'-CTT ^{3'}	5'-gTC ^{3'}
	412	693	363	527			
	5'-ATA ^{3'}	5'-ggT ^{3'}	5'-AgC ^{3'}	5'-TgA ^{3'}			
3'-primer(s) ³	499	419	309	259	728	292	527
	5'-ggT ^{3'}	5'-Cgg ^{3'}	5'-ATC ^{3'}	5'-CTC ^{3'}	5'-CCT ^{3'}	5'-gTA ^{3'}	5'-CCT ^{3'}
		572	463	605			
		5'-gCA ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}			
		755					
		5'-CAA ^{3'}					
Well No.	13	14	15	16	17	18	19

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.

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

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Lot No.: **4H4**

Lot-specific information

CELL LINE VALIDATION SHEET																			
HLA-B*48 SSP primer set²																			
			Prod. No.:	Well															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				201325701	201325702	201325703	201325704	201325705	201325706	201552807	201325708	201325709	201325710	201325711	201325712	201325713	201325714	201325715	201325716
	IHWC cell line¹	B*																	
1	9001 SA	*07:02		-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+
2	9280 LK707	*52:01	*73:01	-	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-
3	9011 E4181324	*52:01		-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
4	9275 GU373	*15:10	*53:01	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*37:01		-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-
6	9353 SM	*39:01	*51:01	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
7	9020 QBL	*18:01		-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
8	9025 DEU	*35:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*38:01		-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-
10	9107 LKT3	*54:01		-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
11	9051 PITOUT	*44:03		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*57:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
13	9025 JESTHOM	*27:05		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*15:01	*15:20	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-
15	9075 DKB	*40:01		+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*40:02		-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
17	9282 CTM3953540	*08:01	*55:01	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
18	9257 32367	*14:01	*56:01	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
19	9038 BM16	*18:01		-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
20	9059 SLE005	*40:01		+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*15:01		-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
22	9056 KOSE	*35:03		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*40:02	*56:02	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
24	9035 JBUSH	*38:01		-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-
25	9049 IBW9	*14:02		-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
26	9285 WT49	*58:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*07:05	*51:01	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	+
28	9320 BEL5GB	*44:02	*44:03	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*44:03		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*42:01		-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
31	9019 DUCAF	*18:01		-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
32	9297 HAG	*41:02		+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
33	9098 MT14B	*40:01		+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*38:01		-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-
35	9302 SSTO	*44:02		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*15:01	*35:01	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-
37	9065 HHKB	*07:02		-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+
38	9099 LZL	*15:01		-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
39	9315 CML	*08:01	*27:05	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
40	9134 WHONP199	*13:02	*46:01	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
41	9055 H0301	*14:02		-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*46:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*46:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*38:01		-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-
45	9239 SHJO	*42:01	*50:01	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
46	9013 SCHU	*07:02		-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+
47	9045 TUBO	*51:01		-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
48	9303 TER-ND	*35:01	*44:03	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

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Lot No.: **4H4**

Lot-specific information

CELL LINE VALIDATION SHEET						
HLA-B*48 SSP primer set²						
				Well		
				17	18	19
				Prod. No.:	201552817	201552818
				201552819		
IHWC cell line¹			B*			
1	9001	SA	*07:02	-	-	-
2	9280	LK707	*52:01	*73:01	-	-
3	9011	E4181324	*52:01		-	-
4	9275	GU373	*15:10	*53:01	-	-
5	9009	KAS011	*37:01		-	-
6	9353	SM	*39:01	*51:01	-	-
7	9020	QBL	*18:01		-	-
8	9025	DEU	*35:01		-	+
9	9026	YAR	*38:01		-	-
10	9107	LKT3	*54:01		-	-
11	9051	PITOUT	*44:03		-	-
12	9052	DBB	*57:01		-	-
13	9025	JESTHOM	*27:05		-	-
14	9071	OLGA	*15:01	*15:20	-	-
15	9075	DKB	*40:01		-	-
16	9037	SWEIG007	*40:02		-	-
17	9282	CTM3953540	*08:01	*55:01	-	-
18	9257	32367	*14:01	*56:01	-	-
19	9038	BM16	*18:01		-	-
20	9059	SLE005	*40:01		-	-
21	9064	AMALA	*15:01		-	-
22	9056	KOSE	*35:03		-	+
23	9124	IHL	*40:02	*56:02	-	-
24	9035	JBUSH	*38:01		-	-
25	9049	IBW9	*14:02		-	-
26	9285	WT49	*58:01		-	-
27	9191	CH1007	*07:05	*51:01	-	-
28	9320	BEL5GB	*44:02	*44:03	-	-
29	9050	MOU	*44:03		-	-
30	9021	RSH	*42:01		-	-
31	9019	DUCAF	*18:01		-	-
32	9297	HAG	*41:02		-	-
33	9098	MT14B	*40:01		-	-
34	9104	DHIF	*38:01		-	-
35	9302	SSTO	*44:02		-	-
36	9024	KT17	*15:01	*35:01	-	+
37	9065	HHKB	*07:02		-	-
38	9099	LZL	*15:01		-	-
39	9315	CML	*08:01	*27:05	-	-
40	9134	WHONP199	*13:02	*46:01	-	-
41	9055	H0301	*14:02		-	-
42	9066	TAB089	*46:01		-	-
43	9076	T7526	*46:01		-	-
44	9057	TEM	*38:01		-	-
45	9239	SHJO	*42:01	*50:01	-	-
46	9013	SCHU	*07:02		-	-
47	9045	TUBO	*51:01		-	-
48	9303	TER-ND	*35:01	*44:03	-	+

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

Lot No.: **4H4**

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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 6, 8 and 17 were available. The specificities of the primers in 6 and 8 primer solutions were tested by separately adding additional 3'-primers, respectively additional 5'-primers. In primer solution 17 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 6 and 8, one or two 5'-primers were not possible to test, and in primer solutions 9 and 14 two 3'-primers were not possible to test. Additional primers in primer solutions 5, 12, 14 and 16 were tested by separately adding one 5'-primer and/or one 3'-primer.

101.546-06 – including *Taq* pol., IFU-01

101.546-06u – without *Taq* pol., IFU-02

Lot No.: **4H4**

Lot-specific information

Visit www.olerup.com for
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