Product Insert

101.543-06 – including Taq pol., IFU-01 **101.543-06u – without Taq pol.,** IFU-02 Visit <u>www.olerup-ssp.com</u> for "Instructions for Use" (IFU)

Lot No.: **05Y**

Lot-specific information Olerup SSP[®] HLA-B*42

Product number:	101.543-06 – including <i>Taq</i> pol. 101.543-06u – without <i>Taq</i> pol.
Lot number:	05Y
Expiry date:	2017-November-01
Number of tests:	6
Number of wells per test:	15+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. 05Y.

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*42 LOT (26V).

The HLA-B*42 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*42 primer set, specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup* SSP[®] HLA-B*42 lot was made (Lot No. 26V). The kit design is based on IMGT/HLA database 3.19.0.

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Lot No.: **05Y**

Lot-specific information

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

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

Well	5'-primer	3'-primer	rationale
14	-	Added	3'-primer added for the B*42:18 allele.
16	-	-	Updated negative control.



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Lot No.: **05Y**

Lot-specific information

Well **16** contains <u>Negative Control primer pairs</u>, 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	105	200	105	80	75	80	85
product							
5'-primer ¹	164	340	440	45	45	43	36
	^{5'} -CAC ^{3'}	^{5'} -Agg ^{3'}	^{5'} -TTA3'	^{5'} -Tgg ^{3'}	^{5'} -Tgg ^{3'}	^{5'} -Tgg ^{3'}	^{5'} -TAC ^{3'}
							36
							^{5'} -TAT ^{3'}
3'-primer ²	231	2 nd I	507	59	58	57	47
- p	^{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′} -TgT³′
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 <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given.

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-

Nucleotide and codon numbering as on the <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given.

determining 3'-end of the primer is given in the anti-sense direction.

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Lot No.: **05Y**

Lot-specific information PRODUCT DESCRIPTION

HLA-B*42 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*42:01 to B*42:20 alleles.

PLATE LAYOUT

Each HLA-B*42 test consists of 16 PCR reactions in a 16 well cut PCR plate.

1	2	3	4 12	5	6	7	8
9	10	11	12	13	14	15	NC

The 16 well PCR plate is marked with 'HLA-B*42' in silver/gray ink.

Well No. 1 is marked with the Lot No. '05Y'.

Wells 1 to 15 – HLA-B*42 high resolution primers.

Well 16 – 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 16 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*42 alleles will be amplified by primer mixes 1 to 12, 14 and 15. In addition, a few HLA-C allele will be amplified by primer mixes 1, 7, 9, 10 and 14. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*42, i.e. **B*42:01 to B*42:20**, recognized by the HLA Nomenclature Committee in January 2015^{1,2} will be amplified by the primers in the HLA-B*42 SSP kit³.

The HLA-B*42 kit enables separation of the confirmed HLA-B*42 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*42 alleles is listed below.

The HLA-B*42 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles

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Lot No.: **05Y**

Lot-specific information

The HLA-B*42 subtyping kit cannot distinguish the silent mutations in the B*42:01:01-42:01:03 and the 42:05:01-42:05:02 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2015-January-19, release 3.19.0, <u>www.ebi.ac.uk/imgt/hla</u>.

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 HLA-B*42 primer set cannot separate the B*42:07 and the B*08:94 alleles. These alleles can be distinguished by the HLA-B low resolution kit and/or the HLA-B*08 high resolution kit.

ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
B*42:01:01	Confirmed	B*42:09	Confirmed	B*42:19	Unconfirmed
B*42:01:02	Unconfirmed	B*42:10	Confirmed	B*42:20	Unconfirmed
B*42:01:03	Unconfirmed	B*42:11	Unconfirmed		
B*42:02	Confirmed	B*42:12	Unconfirmed		
B*42:04	Unconfirmed	B*42:13	Unconfirmed		
B*42:05:01	Confirmed	B*42:14	Unconfirmed		
B*42:05:02	Unconfirmed	B*42:15	Unconfirmed		
B*42:06	Unconfirmed	B*42:16	Unconfirmed		
B*42:07	Unconfirmed	B*42:17	Unconfirmed		
B*42:08	Unconfirmed	B*42:18	Confirmed		

¹Allele status "confirmed" or "unconfirmed" as listed on the IMGT/HLA web page 2015-January-19, release 3.19.0, <u>www.ebi.ac.uk/imgt/hla</u>.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-B*42 homo- and heterozygotes is available upon request.



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Lot No.: **05Y**

Lot-specific information SPECIFICITY TABLE

HLA-B*42 SSP subtyping

Specificities and sizes of the PCR products of the 15+1 primer mixes used for HLA-B*42 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*42 alleles ³	Other amplified HLA Class I alleles ⁴
1	215 bp	800 bp	*42:01:01- 42:01:03, 42:04- 42:06, 42:08, 42:10, 42:12- 42:16, 42:19- 42:20	*07:02:01-07:02:27, 07:02:29-07:02:45, 07:04-07:07, 07:09, 07:11-07:12, 07:14- 07:15, 07:17-07:26, 07:28, 07:30-07:31, 07:33:01-07:36, 07:39-07:49N, 07:51- 07:68:03, 07:70, 07:72-07:82, 07:84, 07:86-07:115, 07:117-07:124, 07:126- 07:142, 07:144-07:179, 07:181N- 07:182N, 07:184-07:185, 07:187-07:206, 07:208-07:218, 07:220-07:227, 07:229- 07:241, 14:21, 15:76, 15:101, 15:255, 35:76, 38:26, 44:90, 54:01:01-54:01:06, 54:03-54:06, 54:08N-54:23, 54:25-54:29, 54:31-54:34, 55:01:01-55:05, 55:07- 55:15, 55:17, 55:19-55:24, 55:26-55:33, 55:35-55:46, 55:48-55:63, 55:65-55:70, 56:01:01:01-56:07, 56:09-56:13, 56:15- 56:16, 56:18-56:22, 56:24-56:32, 56:34, 56:36-56:41, 56:43, 56:45, 67:01:01- 67:01:03, 67:03-67:05, 81:01-81:04N, 81:06-81:07, 82:01-82:03, 83:01, C*14:62
2	215 bp	1070 bp	*42:01:01-42:02, 42:05:01-42:12, 42:14-42:18, 42:20	*07:04, 07:19, 07:25, 07:146, 08:01:01- 08:05, 08:07-08:08N, 08:10-08:11, 08:14- 08:15, 08:17-08:19N, 08:21-08:24, 08:26:01-08:39, 08:41-08:48, 08:50- 08:54, 08:56:01-08:59:02, 08:61-08:69, 08:71-08:78, 08:80-08:83, 08:85-08:88, 08:90-08:106, 08:108-08:128, 08:130- 08:140, 35:87, 37:09, 41:02:01-41:02:06, 41:04, 41:10-41:11, 41:13, 41:15, 41:18- 41:19, 41:23-41:24, 41:27, 41:31, 44:106, 44:158, 44:166, 44:213
3	215 bp	1070 bp	*42:02, 42:09, 42:17-42:18	*07:143, 40:166, 45:06, 54:02, 55:16, 56:35
4	220 bp	1070 bp	*42:04	*08:09, 08:84, 13:46, 15:83, 41:01:01- 41:01:03, 41:05-41:07, 41:09, 41:12, 41:14, 41:16-41:17, 41:20-41:22, 41:25- 41:26, 41:28-41:30, 41:32-41:35, 44:15, 44:18, 44:20, 44:100, 45:01:01-45:15, 51:08:01-51:08:02, 51:20, 51:36, 51:44N, 51:97, 51:141, 51:153, 52:19, 55:20, 55:56, 56:13
5 ⁵	105 bp	800 bp	*42:05:01- 42:05:02	*07:04, 07:25, 07:146, 40:136, 40:231, 40:279, 41:08

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Lot N	o.: 05Y	Lo	t-specific informati	on
6	165 bp 215 bp	1070 bp	*42:01:01-42:02, 42:04-42:08, 42:10-42:15, 42:17-42:18	*08:01:01-08:01:22, 08:01:24-08:05, 08:08N-08:12:03, 08:15-08:19N, 08:21- 08:24, 08:26:01-08:27, 08:29-08:36, 08:38-08:39, 08:41-08:48, 08:50-08:54, 08:56:01-08:69, 08:71-08:73, 08:75- 08:76, 08:78, 08:80-08:86N, 08:88, 08:90- 08:106, 08:109-08:110, 08:112-08:140, 15:83, 35:87, 37:12, 38:30, 40:136, 40:231, 40:279, 41:01:01-41:03:02, 41:05- 41:09, 41:11-41:17, 41:19-41:20, 41:22- 41:35, 44:14, 44:62, 44:166, 44:213, 45:14, 51:08:01-51:08:02, 51:20, 51:36, 51:44N, 51:97, 51:141, 51:153, 52:19, 55:20, 55:56, 56:13, 57:09, 82:01-82:03
7	165 bp	1070 bp	*42:06, 42:09, 42:16, 42:20	*07:04, 07:19, 07:25, 07:146, 08:07, 08:37, 08:49, 08:69, 08:89, 08:107, 13:46, 15:51, 15:179:01-15:179:02, 15:199, 15:218Q, 18:56, 35:38, 35:115, 35:169, 35:260, 37:01:01-37:01:09, 37:03N- 37:06:02, 37:08-37:11, 37:13-37:24, 37:26-37:38, 37:41-37:54, 38:30, 41:04, 41:18, 41:21, 44:15, 44:17-44:18, 44:51, 44:117 ^w , 44:123, 44:140, 45:01:01-45:02, 45:04-45:15, 53:22, 55:56, 56:13, 57:24, 82:01-82:03, C*05:62, C*07:04:01 ^w - 07:04:10 ^w , C*07:11 ^w -07:12 ^w , C*07:45 ^w , C*07:63 ^w , C*07:101 ^w , C*07:139 ^w , C*07:142 ^w , C*07:272 ^w , C*07:323 ^w - 07:324 ^w , C*07:328 ^w -07:329N ^w , C*07:36 ^w , C*07:357 ^w -07:358 ^w , C*07:361 ^w , C*07:394 ^w -07:395 ^w , C*07:403 ^w , C*07:406 ^w , C*15:25
8	215 bp	1070 bp	*42:07, 42:11	*07:03, 07:08, 07:16, 07:27, 07:32, 07:38, 07:50, 07:69, 07:85, 07:180, 07:207, 07:228, 08:94, 13:14, 13:83, 14:01:01- 14:01:05, 14:07N-14:08:02, 14:10, 14:12, 14:14, 14:19, 14:26, 14:32, 14:40, 15:68, 15:71, 15:175, 18:01:01:01-18:01:12, 18:01:14-18:03, 18:05-18:15, 18:17N- 18:24, 18:26-18:67, 18:69, 18:71-18:106, 27:12, 27:16, 27:18, 27:23, 27:29, 27:92, 27:119, 35:50, 35:84, 35:162, 35:197, 35:231, 35:267, 35:269, 37:01:01-37:54, 38:01:01-38:04, 38:06-38:25, 38:27- 38:32, 38:35-38:53, 38:55Q-38:56, 39:01:01:01-39:01:01:03, 39:01:03-39:03, 39:05:01-39:11, 39:13:01-39:20, 39:22- 39:51, 39:53-39:62, 39:64-39:71, 39:73- 39:75, 39:77-39:98, 39:99 ^w , 39:100, 40:02:01-40:06:11, 40:08-40:09, 40:11:01-40:11:02, 40:13, 40:18-40:20, 40:24, 40:26-40:27:01, 40:28-40:29, 40:35:01-40:35:02, 40:37, 40:39-40:40,

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				40:44, 40:50, 40:56, 40:64, 40:68, 40:70:01-40:71, 40:74-40:75, 40:78, 40:82-40:83, 40:85-40:86, 40:89-40:91, 40:93-40:99, 40:104-40:105, 40:107, 40:109, 40:111, 40:115, 40:119-40:120, 40:122, 40:127, 40:131, 40:133Q, 40:142N-40:145, 40:148-40:149, 40:157, 40:159, 40:161-40:162, 40:165, 40:167, 40:169, 40:173-40:174, 40:176-40:177, 40:180-40:181, 40:184, 40:189-40:190, 40:201-40:203, 40:205-40:206, 40:209- 40:211, 40:214, 40:220, 40:224-40:225, 40:229-40:230, 40:232, 40:243-40:244, 40:246, 40:248, 40:254-40:256N, 40:266- 40:271, 40:274-40:276, 40:283-40:284, 40:287, 40:289-40:297, 44:103, 44:130- 44:131, 44:212, 48:01:01-48:21, 48:23- 48:36, 49:31, 51:57, 51:103, 51:116, 53:21
9	170 bp	1070 bp	*42:01:01-42:02, 42:04-42:06, 42:08-42:10, 42:12-42:20	*07:02:01-07:02:27, 07:02:29-07:02:45, 07:04-07:07, 07:09, 07:11-07:12, 07:14- 07:15, 07:17-07:26, 07:28-07:31, 07:33:01-07:36, 07:39-07:49N, 07:51- 07:55, 07:57-07:64, 07:66-07:68:03, 07:70-07:71, 07:73-07:82, 07:84, 07:86- 07:124, 07:126-07:176, 07:178-07:179, 07:181N-07:182N, 07:184-07:205, 07:208-07:218, 07:220-07:227, 07:229- 07:241, 08:123, 08:132, 14:21, 15:255, 38:26, 54:21, 55:10, 56:16, 67:01:01- 67:01:03, 67:03-67:05, 81:01-81:04N, 81:06-81:07, 82:01-82:03, 83:01, C*07:335
10	280 bp	800 bp	*42:08	*07:19, 07:33:01-07:33:03, 07:53, 07:60, 07:100, 08:103, 08:134, 13:71, 14:05, 14:13, 15:07:01-15:07:03, 15:45, 15:55, 15:68, 15:126, 15:207, 15:324, 15:329, 15:331, 18:14, 27:07:01-27:07:04, 27:11, 27:24, 27:32-27:34, 27:43, 35:05:01- 35:05:03, 35:22, 35:31, 35:51, 35:58, 35:72, 35:89, 35:97, 35:114, 35:199, 35:232, 37:09, 38:19, 39:03, 39:14, 39:24:01-39:24:02, 39:29, 39:37, 39:76, 40:02:01-40:03, 40:05, 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,

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Lot No.:	05Y	Lo	t-specific information	on
				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, 44:54, 44:106, 44:135, 44:158, 44:184, 44:213, 46:12, 48:04, 48:13, 48:24, 51:64, 51:81, 51:148, 53:14, 55:04, 55:49, 55:51, 58:18, 58:27, C*02:60, C*03:05, C*03:25, C*03:27, C*03:167, C*07:294, C*08:08:01-08:08:02
11 ⁵	115 bp 180 bp	1070 bp	*42:19 *42:10, 42:17	*08:20, 08:53:01-08:53:02 *08:49, 08:71, 27:83, 41:24, 53:15
12 ⁵	105 bp	1070 bp	*42:11	*07:27, 07:50, 08:04, 08:17, 08:54, 08:110, 15:03:01-15:03:05, 15:47:01- 15:47:02, 15:54, 15:61-15:62, 15:64:01- 15:64:02, 15:68-15:69, 15:91, 15:98, 15:103, 15:123, 15:127, 15:131-15:132, 15:151, 15:156, 15:158, 15:173, 15:210, 15:220, 15:235, 15:242, 15:253, 15:259, 15:266, 15:274, 15:281-15:282, 18:37, 27:18, 27:29, 37:28, 38:03, 39:02:01- 39:02:02, 39:08, 39:13:01-39:13:02, 39:23, 39:39:01-39:39:02, 39:49, 39:88, 40:12, 40:46, 40:93, 44:40, 44:44, 44:130, 44:156, 44:210, 48:01:01-48:05, 48:07- 48:30, 48:32-48:36, 52:16, 55:18, 56:44
13 ⁵	65 bp	1070 bp	*42:12	
14	195 bp	1070 bp	*42:15, 42:18	*07:11, 07:57, 07:75, 07:160, 07:240, 08:16-08:17, 08:47, 08:123, 18:49, 18:79, 37:05, 39:20, C*01:59, C*06:82, C*07:49, C*07:124, C*07:155, C*07:210, C*07:238, C*07:247, C*07:403
45	290 bp	1070 6-	*42:13	*08:49, 08:60, 08:76, 08:129, 53:15
15	230 bp	1070 bp	*42:14	*08:116, 41:06, 41:15
16 ⁶			Negative Control	

¹Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of HLA-B*42 SSP typings.

When the primers in a primer mix can give rise to HLA-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits, the alleles listed are specified according to amplicon length.

Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

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

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

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherit feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR 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.

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Lot No.: **05Y**

Lot-specific information

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*42 alleles will be amplified by primer mixes 1 to 12, 14 and 15. In addition, a few HLA-C allele will be amplified by primer mixes 1, 7, 9, 10 and 14.

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

⁶Primer mix 16 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.: **05Y**

Lot-specific information PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	215	215	215	220	105	165	165	215	170	280	115	105
PCR product											180	
Length of int.	800	1070	1070	1070	800	1070	1070	1070	1070	800	1070	1070
pos. control ¹												
5'-primer(s) ²	97	363	97	357	540	412	412	103	142	363	463	206
	5' -TCT 3'	^{5'} -AgC ^{3'}	5' -TCC 3'	^{5'} -Tgg ^{3'}	5' -gAC 3'	^{5'} -ATA ^{3'}	^{5'} -ATg ^{3'}	5' -CCT 3'	5' -TCT 3'	^{5'} -AgC ^{3'}	^{5'} -TgA ^{3'}	^{5'} -AgA ^{3'}
							419	103			527	
							^{5'} -gTT ^{3'}	5' -CCT 3'			^{5'} -TgA ^{3'}	
							419					
							^{5'} -gTT ^{3'}					
3'-primer(s) ³	272	538	272	538	605	538	538	277	272	603	603	272
. ()	^{5'} -TgT ^{3'}	^{5'} -gTC ^{3'}	^{5'} -TgT ^{3'}	^{5'} -gTC ^{3'}	5' -gCT 3'	5' -gTC 3'	^{5'} -gTC ^{3'}	^{5'} -ggT ^{3'}	^{5'} -TgT ^{3'}	^{5'} -gTC ^{3'}	^{5'} -gTg ^{3'}	^{5'} -Tgg ^{3'}
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15
Length of spec.	65	195	230
PCR product		290	
Length of int.	1070	1070	1070
pos. control ¹			
5'-primer(s) ²	540	142	412
	^{5'} -gAC ^{3'}	^{5'} -TCT ^{3'}	^{5'} -ATA ^{3'}
		355	
		^{5'} -TCA ^{3'}	
3'-primer(s) ³	566	289	603
	5' -CCC 3'	5' -AgC 3'	^{5'} -gTg ^{3'}
		302	
		^{5'} -ggT ^{3'}	
		603	
		^{5'} -gTg ^{3'}	
Well No.	13	14	15

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 <u>www.ebi.ac.uk/imgt/hla</u> 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 <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given.

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				NE VA	LI	DF	۱	IO	Ν	St	1E	E							
			HLA	-B*42 \$	SS	Ρp	orir	ne	r s	et ²									
					1	•						Ne							
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
				_		-		-	-	-		-	-	-					2
				 <u>o</u>	201548801	201548802	201548803	201548804	201548805	201548806	201548807	201548808	201548809	201548810	201548811	201548812	201548813	201548814	
				2	548	548	548	548	548	548	548	548	548	548	548	548	548	548	548
				Prod. No.	01	01	01	01	01	01	01	01	01	01	01	01	01	01	20154881
		/C cell line ¹				N	N	N	~		N	N		N	N		N	~~~	
-	9001		_	B*	-	_							<u> </u>				_		_
1		SA LK707	*07:02	*73:01	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
2		E4181324	*52:01	73.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4		GU373	*15:10	*53:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
5		KAS011	*37:01	00.01	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
6	9353		*39:01	*51:01	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
7	9020		*18:01	0	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
8	9025		*35:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026		*38:01		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
10	9107		*54:01		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11		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		CTM3953540	*08:01	*55:01	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-
18		32367	*14:01	*56:01	+	-	•	-	-	-	-	+	-	-	-	•	-	•	-
19		BM16	*18:01		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
20		SLE005	*40:01		-	•	•	•	-	-	-	-	-	-	•	•	•	•	-
21		AMALA	*15:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22		KOSE	*35:03	*=0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124		*40:02	*56:02	+	-	•	-	-	-	-	+	-	+	-	-	-	•	-
24		JBUSH	*38:01		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
25	9049		*14:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26 27		WT49	*58:01	*51.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
27		CH1007 ¹ BEL5GB	*07:05	*51:01 *44:03	+	-	-	-	-	-	-	-	+	-	-	-	-	-	
20	9050		*44:02	44.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
29 30	9050		*42:01				-	-	-		-	-		-	-	-	-	-	
31		DUCAF	*18:01		+	+	-	-	-	+	-	+	+	-	-	-	-	-	
32	9297		*41:02		-	+	-	-	-	+	-	- -	-	-	-	-	-	-	
33		MT14B	*40:01		-	т -	-	-	-	- -	-	-	-	-	-	-	-	-	
34	9104		*38:01		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
35		SSTO	*44:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36		KT17	*15:01	*35:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37		ННКВ	*07:02		+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
38	9099		*15:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315		*08:01	*27:05	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*13:02	*46:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41		H0301	*14:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*46:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*46:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	•
44	9057	TEM	*38:01		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
45		SHJO	*42:01	*50:01	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-
46		SCHU	*07:02		+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
47		TUBO ¹	*51:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

¹The provided cell line HLA specificities are retrieved from the <u>http://www.ihwg.org/hla</u> web site. The specificity of an individual cell line may thus be subject to change.



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Lot-specific information

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 solution 5, 11 and 13 to 15 were available. The specificities of the primers in primer solution 5, 11 and 14 were tested by separately adding one additional 3'-primer, respectively one additional 5'-primer. In primer solutions 13 and 15 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solution 14 two 3'-primers were not possible to test. One additional 5'-primer in primer solution 7 was tested by separately adding one additional 3'-primer.



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Lot-specific information



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