Visit <u>www.olerup-ssp.com</u> for "Instructions for Use" (IFU)

Lot No.: 4D2 Lot-specific information

Olerup SSP® HLA-B*81

Product number: 101.553-06 – including *Taq* pol.

101.553-06u - without *Taq* pol.

Lot number: 4D2

Expiry date: 2018-07-01

Number of tests: 6 Number of wells per test: 9+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. 4D2.

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*81 Lot (99X).

The HLA-B*81 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.

One well has been added to HLA-B*81, well 10.

A well containing Negative Control primer pairs has been added.

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

The HLA-B*81 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup* SSP® HLA-B*81 lot was made (Lot No. 99X). The kit design is based on IMGT/HLA database 3.22.0.

€

¹As described in section Uniquely Identified Alleles.

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

Lot No.: 4D2 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
4	-	Exchanged	3'-primer exchanged for improved allelic resolution of the B*81 alleles.
5	Modified	-	5'-primers modified for improved HLA-specific amplification.
9	Added	Added	Negative control moved to well 10, primer pair added for the B*81:08 allele.
10	-	-	Negative control added from well 9.

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

Lot No.: 4D2 Lot-specific information

Well **10** 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	105	200	105	80	75	80	85
product							
5'-primer ¹	164	340	440	45	45	43	36
	5'-CAC3'	^{5'} -Agg ^{3'}	^{5'} -TTA3'	⁵ '-Tgg ³ '	^{5'} -Tgg ^{3'}	⁵ '-Tgg ³ '	5'-TAC3'
							36
							^{5'} -TAT ^{3'}
3'-primer ²	231	2 nd I	507	59	58	57	47
•	5'-TgC ^{3'}	⁵ '-AAA ³ '	^{5'} -TTg ^{3'}	5'-CTC3'	^{5'} -ggC ^{3'}	5'-CTC3'	5'-ACA3'
							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 codonnumbering 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.



Visit <u>www.olerup-ssp.com</u> for "Instructions for Use" (IFU)

Lot No.: 4D2 Lot-specific information

PRODUCT DESCRIPTION

HLA-B*81 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for separating the B*81:01 to B*81:08 alleles.

PLATE LAYOUT

Each HLA-B*81 test consists of 10 PCR reactions in a 16 well cut PCR plate. Wells 11 to 16 are empty.

1	2	3	4	5	6	7	8
9	NC	empty	empty	empty	empty	empty	empty

The 16 well PCR plate is marked with 'HLA-B*81' in silver/gray ink.

Well No. 1 is marked with the Lot No. '4D2'.

Wells 1 to 9 – HLA-B*81 high resolution primers.

Well 10 – 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 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*81 alleles will be amplified by primer mixes 1 to 7 and 9. In addition, a few HLA-C alleles will be amplified by primer mixes 4 and 7.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*81 alleles, i.e. **B*81:01 to B*81:08**, recognized by the HLA Nomenclature Committee in October 2015^{1,2} will be amplified by the primers in the HLA-B*81 SSP kit³.

The HLA-B*81 kit enables separation of the confirmed HLA-B*81 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*81 alleles is listed below.

The HLA-B*81 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.



Product Insert Page 5 of 12

101.553-06 – including *Taq* **pol.**, IFU-01 **101.553-06u – without** *Taq* **pol.**, IFU-02

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

Lot No.: 4D2 Lot-specific information

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.

The B*81:02 and the B*07:202 alleles will give rise to identical amplification patterns. These alleles can e.g. be distinguished by the HLA-B low resolution kit and/or HLA-B*07 high resolution kit

ALLELE CONFIRMATION STATUS

Allele	Status ¹
B*81:01	Confirmed
B*81:02	Confirmed
B*81:03	Unconfirmed
B*81:04N	Unconfirmed
B*81:05	Confirmed
B*81:06	Unconfirmed
B*81:07	Unconfirmed
B*81:08	Unconfirmed

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

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-B*81 homo- and heterozygotes is available upon request.



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

Lot No.: 4D2

⊙LERUPSSP® HLA-B*81

Lot-specific information

SPECIFICITY TABLE

HLA-B*81 SSP subtyping

Specificities and sizes of the PCR products of the 9+1 primer mixes used for HLA-B*81 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA- B*81 alleles ³	Other amplified HLA-B alleles ⁴
1	175 bp	800 bp	*81:01-81:08	*07:174, 07:202, 07:222, 27:24, 40:31, 40:45, 40:80, 48:01:01-48:01:06, 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, 48:27-48:37
2	410 bp	1070 bp	*81:01, 81:03 [?] - 81:08 [?]	*07:65, 27:01-27:11, 27:13-27:15, 27:17, 27:19-27:21, 27:24-27:28, 27:30-27:38, 27:40-27:58, 27:60-27:76, 27:78-27:91, 27:93-27:118, 27:120-27:128, 27:130-27:136, 27:138-27:145, 35:76, 35:186, 35:229, 44:90, 44:97, 54:01:01-54:29, 54:31-54:34, 55:01:01-55:05, 55:07-55:17, 55:19-55:76, 56:01:01:01-56:16, 56:18-56:22, 56:24-56:32, 56:34-56:41, 56:43, 56:45-56:49, 82:01-82:03, 83:01
36	405 bp	1070 bp	*81:02, 81:03 [?] - 81:08 [?]	*07:02:01-07:02:48, 07:04-07:07, 07:09-07:15, 07:17-07:26, 07:28-07:31, 07:33:01-07:36, 07:39-07:46, 07:48-07:49N, 07:51-07:64, 07:66-07:68:03, 07:70-07:84, 07:86-07:179, 07:181N-07:206, 07:208-07:218, 07:220-07:227, 07:229-07:231N, 07:233-07:256, 08:123, 08:132, 14:21, 38:26, 42:01:01-42:02:01:02, 42:04-42:06, 42:08-42:10, 42:12-42:21, 45:06, 67:01:01-67:05, 73:01-73:02
4	190 bp	1070 bp	*81:01-81:08	*07:147, 07:151:01-07:151:02, 07:202, 08:120, 40:31, 40:80, 40:287, 41:38, 48:01:01-48:01:06, 48:04:01-48:04:02, 48:06-48:07, 48:09, 48:11, 48:14-48:16, 48:18-48:20, 48:22, 48:24, 48:27-48:37, C*07:294
55	105 bp 165 bp	1070 bp	*81:05 *81:03	*07:83 *15:20, 15:228, 15:305, 35:01:01:01-35:01:27, 35:01:29-35:30, 35:32:01-35:34, 35:36-35:42:02, 35:44-35:45, 35:47-35:56, 35:58-35:59:02, 35:61:01-35:72, 35:74-35:78, 35:80-35:86, 35:88-35:184, 35:186-35:278, 35:280-35:300, 48:02:01-48:02:03, 51:01:01-01-51:24:05, 51:26-51:41N, 51:43-51:46, 51:48-51:156, 51:158:01-51:163, 51:165-51:195, 52:01:01-53:41,



Page 7 of 12

101.553-06 – including *Taq* **pol.**, IFU-01 **101.553-06u – without** *Taq* **pol.**, IFU-02

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

Lot No.: 4D2 Lot-specific information

15:51-15:52, 15:54, 15:62, 15:64:01- 15:64:02, 15:68-15:69, 15:72, 15:74, 15:80, 15:90-15:91, 15:93, 15:98-15:99, 15:103, 15:108, 15:114, 15:123-15:124, 15:127, 15:131-15:134, 15:151, 15:153, 15:156, 15:158, 15:161, 15:173, 15:176, 15:186, 15:197-15:198, 15:200, 15:210, 15:220-15:221, 15:226N, 15:229, 15:235, 15:238, 15:242-15:243, 15:252- 15:253, 15:259, 15:263, 15:266, 15:274- 15:275:02, 15:281-15:282, 15:290, 15:292-15:293, 15:306-15:307, 15:311- 15:314, 15:323, 15:329, 15:335, 15:337- 15:338, 15:351, 15:354, 15:369, 18:37, 27:18, 27:23, 27:44, 27:131, 35:25- 35:26, 35:49, 39:05:01-39:05:02, 39:07- 39:08, 39:11, 39:13:01-39:13:02, 39:20,	LOT NO			t-specific information	211
7 165 bp *81:05 *07:83 *07:10, 37:51, 38:11, 48:36, 54:02, 67:02, C*03:137, C*03:182, C*07:102*, C*07:351, C*12:82 8 140 bp 1070 bp *81:07 9 125 bp 1070 bp *81:08 *07:16, 07:37, 07:50, 07:75, 07:91, 07:207, 07:228, 08:23, 08:54, 08:110, 14:14, 14:18, 15:03:01:01-15:03:05, 15:09-15:10:04, 15:18:01-15:18:06, 15:29, 15:37, 15:47:01-15:47:02, 15:49, 15:51-15:52, 15:54, 15:62, 15:64:01-15:64:02, 15:68-15:69, 15:72, 15:74, 15:80, 15:90-15:91, 15:93, 15:93-15:99, 15:103, 15:108, 15:114, 15:123-15:124, 15:127, 15:131-15:134, 15:151, 15:153, 15:156, 15:158, 15:161, 15:173, 15:176, 15:186, 15:197-15:198, 15:200, 15:210, 15:220-15:221, 15:226N, 15:229, 15:235, 15:238, 15:242-15:243, 15:252-15:253, 15:259, 15:263, 15:266, 15:274, 15:334, 15:334, 15:337, 15:338, 15:331, 15:334, 15:339, 15:336, 18:337, 27:18, 27:23, 27:44, 27:131, 35:25-35:26, 35:49, 39:05:01-39:05:02, 39:07-39:08, 39:11, 39:13:01-39:13:02, 39:20, 39:37, 39:49, 39:55-39:56, 40:12, 40:46, 40:93, 42:11, 48:01:01-48:37, 55:18, 55:31, 56:23, 56:42, 56:44					58:01:01:01-58:02, 58:04-58:10N, 58:12-58:19, 58:21-58:29, 58:31N-
\$ 140 bp 1070 bp *81:07 \$ 125 bp 1070 bp *81:08 **07:16, 07:37, 07:50, 07:75, 07:91, 07:207, 07:228, 08:23, 08:54, 08:110, 14:14, 14:18, 15:03:01:01-15:03:05, 15:09-15:10:04, 15:18:01-15:14:06, 15:29, 15:37, 15:47:01-15:47:02, 15:49, 15:51-15:52, 15:54, 15:62, 15:64:01-15:64:02, 15:68-15:69, 15:72, 15:74, 15:80, 15:19, 15:13, 15:103, 15:108, 15:114, 15:133, 15:108, 15:114, 15:133, 15:108, 15:14, 15:153, 15:156, 15:158, 15:161, 15:173, 15:176, 15:186, 15:197-15:198, 15:200, 15:210, 15:220-15:221, 15:2261, 15:229, 15:235, 15:238, 15:236, 15:239, 15:336, 15:337, 15:338, 15:351, 15:354, 15:354, 15:354, 15:357, 15:338, 15:351, 15:354, 15:359, 15:338, 15:351, 15:354, 15:359, 15:238, 15:263, 35:259, 39:27-39:08, 39:11, 39:13:01-39:05:02, 39:07-39:08, 39:11, 39:13:01-39:05:02, 39:07-39:08, 39:11, 39:13:01-39:05:02, 39:07-39:08, 39:11, 39:13:01-39:05:02, 39:07-39:08, 39:11, 39:13:01-39:05:02, 39:07-39:08, 39:11, 39:13:01-39:15:02, 39:20, 39:37, 39:49, 39:55-39:56, 40:12, 40:46, 40:93, 42:11, 48:01:01-48:37, 55:18, 55:31, 56:23, 56:42, 56:44	6 ⁵	•	1070 bp		*07:83
95 125 bp 1070 bp *81:08 *07:16, 07:37, 07:50, 07:75, 07:91, 07:207, 07:228, 08:23, 08:54, 08:110, 14:14, 14:18, 15:03:01:01-15:03:05, 15:09-15:10:04, 15:18:01-15:18:06, 15:29, 15:37, 15:47:01-15:47:02, 15:49, 15:51-15:52, 15:54, 15:62, 15:64:01-15:64:02, 15:68-15:69, 15:72, 15:74, 15:80, 15:90-15:91, 15:93, 15:98-15:99, 15:103, 15:108, 15:114, 15:123-15:124, 15:127, 15:134-15:134, 15:151, 15:153, 15:156, 15:158, 15:161, 15:173, 15:176, 15:186, 15:197-15:198, 15:200, 15:210, 15:220-15:221, 15:226N, 15:229, 15:235, 15:238, 15:242-15:243, 15:252-15:235, 15:259, 15:263, 15:266, 15:274-15:275:02, 15:290, 15:292-15:293, 15:306-15:307, 15:311-15:314, 15:323, 15:354, 15:369, 18:37, 27:18, 27:23, 27:44, 27:131, 35:25-35:26, 35:49, 39:05:01-39:05:02, 39:07-39:08, 39:11, 39:13:01-39:13:02, 39:20, 39:37, 39:49, 39:55-39:56, 40:12, 40:46, 40:93, 42:11, 48:01:01-48:37, 55:18, 55:31, 56:23, 56:42, 56:44	7	165 bp	1070 bp	*81:06	54:02, 67:02, C*03:137, C*03:182,
07:207, 07:228, 08:23, 08:54, 08:110, 14:14, 14:18, 15:03:01:01-15:03:05, 15:09-15:10:04, 15:18:01-15:18:06, 15:29, 15:37, 15:47:01-15:47:02, 15:49, 15:51-15:52, 15:54, 15:62, 15:64:01-15:64:02, 15:68-15:69, 15:72, 15:74, 15:80, 15:90-15:91, 15:93, 15:98-15:99, 15:103, 15:108, 15:114, 15:123-15:124, 15:127, 15:131-15:134, 15:151, 15:153, 15:156, 15:158, 15:161, 15:173, 15:176, 15:186, 15:221, 15:226N, 15:229, 15:235, 15:223, 15:226N, 15:229, 15:235, 15:223, 15:2263, 15:266, 15:274-15:275:02, 15:281, 15:282, 15:290, 15:292-15:293, 15:306-15:307, 15:311-15:314, 15:323, 15:329, 15:335, 15:337, 15:338, 15:351, 15:354, 15:369, 18:37, 27:18, 27:23, 27:44, 27:131, 35:25-35:26, 35:49, 39:05:01-39:05:02, 39:07-39:08, 39:11, 39:13:01-39:13:02, 39:20, 39:37, 39:49, 39:55-39:56, 40:12, 40:46, 40:93, 42:11, 48:01:01-48:37, 55:18, 55:31, 56:23, 56:42, 56:44	8	140 bp	1070 bp	*81:07	
10 ⁷ - Negative Control	95	125 bp	1070 bp		07:207, 07:228, 08:23, 08:54, 08:110, 14:14, 14:18, 15:03:01:01-15:03:05, 15:09-15:10:04, 15:18:01-15:18:06, 15:29, 15:37, 15:47:01-15:47:02, 15:49, 15:51-15:52, 15:54, 15:62, 15:64:01-15:64:02, 15:68-15:69, 15:72, 15:74, 15:80, 15:90-15:91, 15:93, 15:98-15:99, 15:103, 15:108, 15:114, 15:123-15:124, 15:127, 15:131-15:134, 15:151, 15:153, 15:156, 15:158, 15:161, 15:173, 15:176, 15:186, 15:197-15:198, 15:200, 15:210, 15:220-15:221, 15:226N, 15:229, 15:235, 15:238, 15:242-15:243, 15:252-15:253, 15:259, 15:263, 15:266, 15:274-15:275:02, 15:281-15:282, 15:290, 15:335, 15:337-15:338, 15:351, 15:354, 15:369, 18:37, 27:18, 27:23, 27:44, 27:131, 35:25-35:26, 35:49, 39:05:01-39:05:02, 39:07-39:08, 39:11, 39:13:01-39:13:02, 39:20, 39:37, 39:49, 39:55-39:56, 40:12, 40:46, 40:93, 42:11, 48:01:01-48:37, 55:18,
	10 ⁷	-	-	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*81 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.



Product Insert Page 8 of 12

101.553-06 – including *Taq* **pol.**, IFU-01 **101.553-06u – without** *Taq* **pol.**, IFU-02

Visit <u>www.olerup-ssp.com</u> for "Instructions for Use" (IFU)

Lot No.: 4D2 Lot-specific information

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*81 alleles will be amplified by primer mixes 1 to 7 and 9. In addition, a few HLA-C alleles will be amplified by primer mixes 4 and 7.

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

⁶Primer mix 3 may have tendency of unspecific amplification.

⁷Primer mix 10 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.

"?", the nucleotide sequence of the primer matching region is not known.



Page 9 of 12

101.553-06 - including Taq pol., IFU-01 **101.553-06u – without Taq pol.**, IFU-02

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

Lot No.: 4D2

Lot-specific information

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9
Length of spec.	175	410	405	190	105	80	165	140	125
PCR product					165	105			
Length of int.	800	1070	1070	1070	1070	1070	1070	1070	1070
pos. control ¹									
5'-primer(s) ²	363	41	44	363	206	206	103	363	206
	^{5'} -AgC ^{3'}	^{5'} -CTg ^{3'}	^{5'} -ggC ^{3'}	^{5'} -AgC ^{3'}	^{5'} -AgA ^{3'}	^{5'} -AgA ^{3'}	5' -CCT 3'	^{5'} -AgC ^{3'}	^{5'} -AgA ^{3'}
					652		103		
					^{5'} -CCg ^{3'}		5' -CCT 3'		
3'-primer(s) ³	499	282	282	512	272	244	226	461	292
	^{5'} -ggA ^{3'}	^{5'} -gCC ^{3'}	^{5'} -gCC ^{3'}	5' -CCA 3'	^{5'} -TgA ^{3'}	^{5'} -CTA ^{3'}	5' -CAC 3'	^{5'} -gCT ^{3'}	^{5'} -gTA ^{3'}
					774	272			
					^{5'} -ggT ^{3'}	^{5'} -TgA ^{3'}			
Well No.	1	2	3	4	5	6	7	8	9

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.



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

Lot No.: 4D2 Lot-specific information

	CELL LINE VALIDATION SHEET												
		_	B*81 S		_		_						
									Nel	I			
					1	2	3	4	5	6	7	8	9
				Prod. No.:	201548301	201548302	201548303	201561904	201561905	201548306	201548307	201548308	201561909
	IHV	C cell line1		B*		- 1	- ,	- 1	- 1	- ,	- ,		H
1	9001		*07:02		-	-	+	-	-	-	-	-	-
2		LK707	*52:01	*73:01	-	-	+	-	+	-	-	-	-
3		E4181324	*52:01	70.01	-	-	-	-	+	-	-	-	-
4		GU373	*15:10	*53:01	-	-	-	-	+	-	-	-	+
5		KAS011	*37:01	33.01	-	-	-	-	-	-	-	-	-
6	9353		*39:01	*51:01	-	-	-	-	+	-	-	-	-
7	9020		*18:01	51.01	-	-	-	-	-	-	-	-	_
8	9025		*35:01		-	-	-	-	+	-	-	-	-
9	9026		*38:01		_	-	-	-		-	-	-	-
10	9107		*54:01		-	+	-	-		-	-		
11		PITOUT	*44:03	-	Ė	+	-	÷	÷	-	-	÷	H
12					<u>-</u>		-			÷		÷	-
12	9052	JESTHOM	*57:01		ļ <u>. </u>		-	-	-		-	-	\vdash
-		OLGA	*27:05	*45.00	-	+		Ė	÷	-	·	-	-
14			*15:01	*15:20	-	-		·	+	-	·	-	-
15	9075		*40:01		-	-	-	-	Ė	-	-	-	-
16		SWEIG007	*40:02	*FF 04	-	-	-	-	-	-	-	-	-
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		-	-	-	-	+	-	-	-	-
23	9124		*40:02	*56:02	-	+	-	-	•	-	-	-	-
24		JBUSH	*38:01		-	-	-	-	•	-	-	-	-
25		IBW9	*14:02		-	-	-	-	•	-	-	-	-
26		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		L-	-	+	-	-	-		-	
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		*08:01	*27:05	-	+	-	-	-	-	-	-	-
40		WHONP199	*13:02	*46:01	-	-	-	-	-	-	-	-	-
41		H0301	*14:02		-	-	-	-	-	-	-	-	口
42		TAB089	*46:01		-	-	-	-	-	-	-	-	-
43		T7526	*46:01		-	-	-	-	-	-	-	-	
44	9057		*38:01		-	-	-	-	-	-	-	-	-
45		SHJO	*42:01	*50:01	-	-	+	-	-	-	-	-	
46		SCHU	*07:02	30.01	-	÷	+	÷	÷	÷	-	-	
40		TUBO	*51:01		-	÷	-	-	+	-	-	÷	
				*44.00	Ē			Ė			Ė		
48	9303	TER-ND	*35:01	*44:03	-	•	-	-	+	-	<u> </u>	•	

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.



Product Insert

Page 11 of 12

101.553-06 – including *Taq* **pol.**, IFU-01 **101.553-06u – without** *Taq* **pol.**, IFU-02

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

Lot No.: 4D2 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 solutions 6, 7 and 8 were available. The specificity of the primers in primer solution 7 were tested by separately adding one additional 5'-primer, respectively two additional 3'-primers. In primer solutions 6 and 8 it was only possible to test the 5'-primer, the 3'-primers were not possible to test. One 3'-primer in primer solution 5 was not possible to test. In addition, one 5'-primer in primer solution 5 was tested by separately adding an additional 3'-primer.



●LERUPSSP®HLA-B*81 Product Insert Page 12 of 12

101.553-06 – including *Taq* **pol.**, IFU-01 **101.553-06u – without** *Taq* **pol.**, IFU-02

Visit <u>www.olerup-ssp.com</u> for "Instructions for Use" (IFU)

Lot No.: 4D2 Lot-specific information

Addresses:

Manufacturer:

Olerup SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.

Tel: +46-8-717 88 27 **Fax:** +46-8-717 88 18

E-mail: info-ssp@olerup.com

Web page: http://www.olerup-ssp.com

Distributed by:

Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.

Tel: +43-1-710 15 00 **Fax:** +43-1-710 15 00 10

E-mail: support-at@olerup.com **Web page:** http://www.olerup.com

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382

Tel: 1-877-OLERUP1 **Fax:** 610-344-7989

E-mail: info.us@olerup.com

Web page: http://www.olerup.com

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.

