●LERUPSSP®HLA-B*51:11N Product Insert Page 1 of 8

101.851-12 – including *Taq* polymerase 101.851-12u – without *Taq* polymerase

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Lot No.: 35V Lot-specific Information www.olerup-ssp.com

Olerup SSP® HLA-B*51:11N

Product number: 101.851-12 – including *Taq* polymerase

101.851-12u - without Taq polymerase

Lot number: 35V

Expiry date: 2016-August-01

Number of tests: 12 Number of wells per test: 2+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. 35V.

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*51:11N Lot (96R)

A well containing Negative Control primer pairs has been added.

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

One well has been added to HLA-B*51:11N, well 3.

The HLA-B*51:11N specificity and interpretation tables have been updated compared the previous *Olerup* SSP® HLA-B*51:11N lot **(Lot No. 96R)**.

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
3	-	-	Negative Control

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Well **3** contains Negative Control primer pairs, that will amplify more than 95% of the Olerup SSP® HLA Class I, DRB, DQB1 and DPB1 amplicons as well as amplicons generated by a control primer pair.

PCR product sizes range from 75 to 430 base pairs.

The PCR product generated by the control primer pair is 430 base pairs.

Length of PCR	105	200	105	80	75	80
product						
5'-primer ¹	164	340	440	45	45	43
	^{5'} -CAC ^{3'}	⁵ '-Agg ³ '	^{5'} -TTA ^{3'}	^{5'} -Tgg ^{3'}	^{5'} -Tgg ^{3'}	⁵ '-Tgg ^{3'}
3'-primer ²	231	2 nd I	507	59	58	57
_	^{5'} -TgC ^{3'}	^{5'} -AAA ^{3'}	^{5'} -TTg ^{3'}	^{5'} -CTC ^{3'}	^{5'} -ggC ^{3'}	^{5'} -CTC ^{3'}
A*	+	+	+			
B*	+	+	+			
C*	+	+	+			
DRB1				+	+	
DRB3				+	+	
DRB5				+		
DQB1					+	
DPB1						+

¹The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

²The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

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PRODUCT DESCRIPTION

HLA-B*51:11N SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-B*51:11N allele.

PLATE LAYOUT

Each test consists of 3 PCR reactions in an 8 well cut PCR plate. Wells 4 to 8 are empty.

1 2 NC empty empty empty empty

The 8 well cut PCR plate is marked with the Lot No. '35V' in silver/gray ink.

Well No. 1 is marked with the Lot No. '35V'.

Wells 1 to 2– HLA-B*51:11N high resolution primers.

Well 3 - 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 8 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*51:11N alleles will be amplified by primer mix 1.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

The HLA-B*51:11N allele will give rise to a unique amplification pattern by the primers in the HLA-B*51:11N kit^{1,2}.

¹HLA-B alleles listed on the IMGT/HLA web page 2013-October-11, release 3.14.0, www.ebi.ac.uk/imgt/hla.

²Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page http://hla.alleles.org/alleles/deleted.html.

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SPECIFICITY TABLE

HLA-B*51:11N SSP subtyping

Specificities and sizes of the PCR products of the 2+1 primer mixes used for HLA-B*51:11N SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B alleles
1 ³	95 bp	800 bp	*07:65 ^w , 07:134 ^w , 08:32, 18:01:01:01-18:11,
			18:13-18:15, 18:17N-18:36, 18:38-18:47,
			18:49-18:65, 18:67-18:94N, 35:01:01:01-
			35:08:04, 35:08:06-35:09:03, 35:11:01-
			35:12:03, 35:14:01-35:15, 35:17:01-35:18,
			35:20:01-35:24:02, 35:27, 35:29:01-35:45,
			35:48, 35:50-35:62, 35:64:01-35:68:02,
			35:70-35:72, 35:74-35:75, 35:76 ^w , 35:77-
			35:79, 35:81-35:153, 35:155-35:185, 35:187-
			35:190, 35:192-35:197, 35:199-35:206,
			35:208-35:209, 35:211-35:216N, 35:218-
			35:225, 35:227-35:240, 37:08, 38:06-38:07,
			39:19:01-39:19:02, 44:06, 51:01:01-51:24:05,
			51:26-51:46, 51:48-51:103, 51:105-51:111,
			51:113-51:117, 51:119-51:146, 51:148-
			51:154, 53:01:01-53:16, 53:18-53:27, 53:29,
	40=1	40-01	53:31, 56:06 ^w , 78:01:01-78:04, 78:07
2	495 bp	1070 bp	*51:11N
3 ⁴			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*51:11N SSP typings.

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. 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.

³HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁴Primer mix 3 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by control primer pairs. PCR product sizes range from 75 to 200 base pairs. The PCR product generated by the control primer pair is 430 base pairs. 'w', might be weakly amplified.

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101.851-12 – including *Taq* polymerase 101.851-12u – without *Taq* polymerase

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PRIMER SPECIFICATION

Well No.	1	2
Length of spec.	95	495
PCR product		
-		
Length of int.	800	1070
pos. control ¹		
5'-primer(s) ²	206	3 rd I
	^{5'} -gAC ^{3'}	^{5'} -CTT ^{3'}
3'-primer(s) ³	259	621
	^{5'} -gTT ^{3'}	^{5'} -ggg ^{3'}
Well No.	1	2

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.

⊙LERUPSSP® HLA-B*51:11N

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CELL LINE VALIDATION SHEET						
	Н	ILA-B*51:1	1N SSI	P kit ²		
					W	ell
					1	2
				Lot No.:	201189901	201189902
	IHW	/C cell line ¹	HL	HLA-B		
1	9001		*07:02		-	-
2	9280	LK707	*52:01	*73:01	-	-
3	9011	E4181324	*52:01		-	-
4	9275	GU373	*15:10	*53:01	+	-
5		KAS011	*37:01		-	-
6	9353		*39:01	*51:01	+	-
7	9020		*18:01		+	-
8	9025		*35:01		+	-
9		YAR	*38:01		-	-
10		LKT3	*54:01		-	-
11		PITOUT	*44:03		H-	Ť
12	9052		*57:01			E
13		JESTHOM	*27:05			÷
				*45.00	-	Ē
14		OLGA	*15:01	*15:20	-	-
15	9075		*40:01		_	-
16	9037		*40:02		-	-
17		CTM3953540	*08:01	*55:01	-	-
18	9257		*14:01	*56:01	-	-
19		BM16	*18:01		+	-
20		SLE005	*40:01		-	•
21		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		MOU	*44:03		-	-
30	9021		*42:01		-	-
31		DUCAF	*18:01		+	-
32		HAG	*41:02		-	-
33		MT14B	*40:01		-	-
34		DHIF	*38:01		-	-
35		SSTO	*44:02		-	-
36		KT17	*15:01	*35:01	+	÷
37		HHKB	*07:02	55.01	T .	
_			*15:01		<u> </u>	Ē
38	9099			*27,05	-	Ē
39	9315		*08:01	*27:05	-	Ē
40		WHONP199	*13:02	*46:01	<u> </u>	-
41		H0301	*14:02		-	_
42		TAB089	*46:01		-	_
43		T7526	*46:01		-	-
44	9057		*38:01		-	_
45		SHJO	*42:01	*50:01	-	-
46	9013	SCHU	*07:02		-	-
47	9045	TUBO	*51:01		+	-
48	9303	TER-ND	*35:01	*44:03	+	-

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OLERUPSSP® HLA-B*51:11N

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

The 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.

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