101.843-12 – including *Taq* polymerase, IFU-01
101.843-12u – without *Taq* polymerase, IFU-02

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Lot No.: 6E6 **Lot-specific information**

Olerup SSP® HLA-A*23 Add-on

Product number:	**101.843-12 – including *Taq* polymerase** **101.843-12u – without *Taq* polymerase**
Lot number:	**6E6**
Expiry date:	**2019-06-01**
Number of tests:	**12**
Number of wells per test:	**3+1**
Storage - pre-aliquoted primers:	**dark at -20ºC**
- PCR Master Mix:	**-20ºC**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 6E6.

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-A*23 Add-on LOT (8D6)

The HLA-A*23 Add-On specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup* SSP® HLA-A*23 Add-On lot was made **(Lot No. 8D6)**. The kit design is based on IMGT/HLA database 3.25.0.

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

The HLA-A*23 Add-on primer set is unchanged compared to the previous *Olerup* SSP® HLA-A*23 Add-on **(Lot No. 8D6)**.

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Well **4** 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'-primer[1]	**164**	**340**	**440**	**45**	**45**	**43**	**36**
	5'-CAC3'	**5'-Agg3'**	**5'-TTA3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-TAC3'**
							36
							5'-TAT3'
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**	**47**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**	**5'-ACA3'**
							48
							5'-gCA3'
							48
							5'-gCC3'
							52
							5'-TgT3'
A*	+	+	+				
B*	+	+	+				
C*	+	+	+				
DRB1				+	+		
DRB3				+	+		
DRB5				+			
DQB1					+		
DPB1						+	
DQA1							+

[1]The 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.

[2]The 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-A*23 Add-on SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for separating the HLA-A*23:17 from the A*23:01 alleles.

PLATE LAYOUT

Each test consists of 4 PCR reactions in an 8 well cut PCR plate. Wells 5 to 8 are empty.

1	2	3	NC	empty	empty	empty	empty

The 8 well cut PCR plate is marked with the Lot No. '6E6' in silver/gray ink.
Well No. 1 is marked with the Lot No. '6E6'.
Wells 1 to 3 – HLA-A*23 Add-on high resolution primers.
Well 4 – 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-A alleles some non-HLA-A*23 alleles will be amplified by primer mixes 1 to 3. In addition, one HLA-B allele will be amplified by primer mix 1.
For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

The HLA-A*23:01 and 23:17 alleles give different amplification patterns in the HLA-A*23 Add-on kit[1,2].

The HLA-A*23 add-on kit cannot distinguish the silent mutations in the A*23:01:01-23:01:15 and 23:01:17-23:01:19 alleles.

[1]Based on HLA-A alleles listed on the IMGT/HLA web page 2016-July-14, release 3.25.0, www.ebi.ac.uk/imgt/hla.
[2]Alleles 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-A*23 Add-on SSP subtyping

Specificities and sizes of the PCR products of the 3+1 primer mixes used for HLA-A*23 Add-on SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified HLA-A*23 alleles[3]	Other amplified HLA-A alleles[4]
1	210 bp	**800 bp**	*23:01:01-23:01:19, 23:03:01-23:65, 23:67-23:68, 23:70-23:74	*02:17:01-02:17:03, 02:108, 02:110, 02:268, 02:300, 02:303, 02:617, 24:13:01-24:13:02, 24:18, 24:24, 24:94, 24:188, 24:207, 24:228, 24:315, 29:07, 29:49, 31:29, **B*18:27**
2[5]	90 bp, 235 bp	1070 bp	*23:01:01-23:01:19, 23:02?-23:04?, 23:05-23:07N, 23:08N?, 23:09, 23:10?-23:16?, 23:18?, 23:19N, 23:20?-23:25?, 23:26, 23:27?-23:37:02?, 23:38N, 23:39?-23:68?, 23:70?-23:74?	*24:25
3[5]	110 bp	1070 bp	*23:01:01-23:01:15, 23:01:16w, 23:01:17-23:01:19, 23:02w, 23:04-23:13, 23:14:01w, 23:14:02-23:23, 23:25-23:33, 23:35-23:56, 23:58-23:65, 23:67-23:68, 23:71-23:74	*02:40:01, 02:40:02w, 02:51, 02:130, 24:24, 24:315w, 31:67-31:68, 32:28, 32:66, 33:32:01, 68:51w
4[6]	-	-	**Negative Control**	

[1]Alleles 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-A*23 Add-on 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.

[2]The 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

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decreases.

[3]For 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.

[4]Due to the sharing of sequence motifs between HLA-A alleles some non-HLA-A*23 alleles will be amplified by primer mixes 1 to 3. In addition, primer mix 1 will amplify the B*18:27 allele.

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

[6]Primer mix 4 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.

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PRIMER SPECIFICATION

Well No.	1	2	3
Length of spec.	210	90	110
PCR product		235	
Length of int.	800	1070	1070
pos. control[1]			
5'-primer(s)[2]	368	28	453
	5' -gTT 3'	5' -TCg 3'	5' -AAA 3'
		920	
		5' -CCA 3'	
3'-primer(s)[3]	539	92	524
	5' -TCA 3'	5' -AAC 3'	5' -CAC 3'
		971	
		5' -CAg 3'	
Well No.	1	2	3

[1]The 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.

[2]The 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.

[3]The nucleotide position matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

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CELL LINE VALIDATION SHEET

HLA-A*23 Add-on SSP kit[2]

					Well		
					1	2	3
				Prod. No.:	201674801	201674802	201674803
	IHWC cell line[1]		A*	A*			
1	9001	SA	*24:02		-	-	-
2	9280	LK707	*02:01		-	-	-
3	9011	E4181324	*01:01		-	-	-
4	9275	GU373	*30:01		-	-	-
5	9009	KAS011	*01:01		-	-	-
6	9353	SM	*02:01	*26:03	-	-	-
7	9020	QBL	*26:01		-	-	-
8	9025	DEU	*31:01		-	-	-
9	9026	YAR	*26:01		-	-	-
10	9107	LKT3	*24:02		-	-	-
11	9051	PITOUT	*29:02		-	-	-
12	9052	DBB	*02:01		-	-	-
13	9004	JESTHOM	*02:01		-	-	-
14	9071	OLGA	*31:01		-	-	-
15	9075	DKB	*24:02		-	-	-
16	9037	SWEIG007	*29:02		-	-	-
17	9282	CTM3953540	*03:01	*80:01	-	-	-
18	9257	32367	*33:03	*74:01	-	-	-
19	9038	BM16	*02:01		-	-	-
20	9059	SLE005	*02:01		-	-	-
21	9064	AMALA	*02:17		+	-	-
22	9056	KOSE	*02:01		-	-	-
23	9124	IHL	*02:01	*34:01	-	-	-
24	9035	JBUSH	*32:01		-	-	-
25	9049	IBW9	*33:01		-	-	-
26	9285	WT49	*02:05		-	-	-
27	9191	CH1007	*24:10	*29:01	-	-	-
28	9320	BEL5GB	*02:01	*29:02	-	-	-
29	9050	MOU	*29:02		-	-	-
30	9021	RSH	*30:01	*68:02	-	-	-
31	9019	DUCAF	*30:02		-	-	-
32	9297	HAG	*02:01		-	-	-
33	9098	MT14B	*31:01		-	-	-
34	9104	DHIF	*31:01		-	-	-
35	9302	SSTO	*32:01		-	-	-
36	9024	KT17	*02:06	*11:01	-	-	-
37	9065	HHKB	*03:01		-	-	-
38	9099	LZL	*02:17		+	-	-
39	9315	CML	*01:01	*03:01	-	-	-
40	9134	WHONP199	*02:07	*30:01	-	-	-
41	9055	H0301	*03:01		-	-	-
42	9066	TAB089	*02:07		-	-	-
43	9076	T7526	*02:06	*02:07	-	-	-
44	9057	TEM	*66:01		-	-	-
45	9239	SHJO	*23:01	*24:02	+	+	+
46	9013	SCHU	*03:01		-	-	-
47	9045	TUBO	*02:16	*03:01	-	-	-
48	9303	TER-ND	*02:01	*11:01	-	-	-

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[1]The 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.

[2]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.
In primer solution 2 one 3'-primer was not possible to test. One additional 5'-primer in primer solution 2 was tested by separately adding one 3'-primer.

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