

101.846-12 – including *Taq* polymerase, IFU-01
101.846-12u – without *Taq* polymerase, IFU-02

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

Lot No.: **3F2**

Lot-specific information

Olerup SSP[®] HLA-A*24 Add-on

Product number:	101.846-12 – including <i>Taq</i> polymerase 101.846-12u – without <i>Taq</i> polymerase
Lot number:	3F2
Expiry date:	2020-01-01
Number of tests:	12
Number of wells per test:	3+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. 3F2.

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*24 ADD-ON LOT (8E0)**

The HLA-A*24 Add-on specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP[®] HLA-A*24 Add-on* lot was made (**Lot No. 8E0**). The kit design is based on IMGT/HLA database 3.26.0

The HLA-A*24 Add-on primer set is unchanged compared to the previous *Olerup SSP[®] HLA-A*24 Add-on* (**Lot No. 8E0**).

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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'-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.

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PRODUCT DESCRIPTION

HLA-A*24 Add-on SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for separating the HLA-A*24:09N and A*24:11N from the A*24:02:01G 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
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The 8 well cut PCR plate is marked with the Lot No. '3F2' in silver/gray ink.

Well No. 1 is marked with the Lot No. '3F2'.

Wells 1 to 3 – HLA-A*24 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*24 alleles will be amplified by some primer mixes. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

The HLA-A*24:09N, 24:11N and A*24:02:01G alleles give different amplification patterns in the HLA-A*24 Add-on kit^{1,2}.

¹Based on HLA-A alleles listed on the IMGT/HLA web page 2016-October-14, release 3.26.0, 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-A*24 Add-on SSP subtyping

Specificities and sizes of the PCR products of the 3+1 primer mixes used for HLA-A*24 Add-on SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*24 alleles ³	Other amplified HLA-A alleles
1	370 bp	800 bp	*24:09N	
2⁴	470 bp	1070 bp	*24:11N	*01:04N, 03:21N, 11:21N, 23:07N
3	175 bp, 205 bp	1070 bp	*24:02:01:01-24:11N, 24:13:01-24:13:02, 24:17-24:50, 24:54-24:56, 24:58-24:63, 24:66-24:91, 24:93, 24:95-24:113, 24:115-24:137, 24:139-24:187, 24:189-24:210, 24:212-24:221, 24:223-24:227, 24:229-24:290, 24:292-24:295, 24:297-24:303N, 24:305-24:315, 24:317-24:323N, 24:325-24:341, 24:343-24:351, 24:354-24:367	*02:17:01 ^w -02:17:03 ^w , 11:139, 23:14:01-23:14:02, 26:16, 33:19, 33:119, 68:45, 68:117
4⁵	-	-	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-A*24 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 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.

⁴Primer mix 2 may give rise to a lower yield of HLA-specific PCR product than the other HLA-A*24 Add-on primer mixes.

⁵Primer 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.

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PRIMER SPECIFICATION

Well No.	1	2	3
Length of spec.	370	470	175
PCR product			205
Length of int.	800	1070	1070
pos. control ¹			
5'-primer(s) ²	742	3 rd I	98
	5' -ACT 3'	5' -ATA 3'	5' -CTC 3'
			368
			5' -gTT 3'
3'-primer(s) ³	971	621	259
	5' -CAg 3'	5' -ggg 3'	5' -gTT 3'
			502
			5' -CTT 3'
			539
			5' -TCT 3'
Well No.	1	2	3

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.

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CELL LINE VALIDATION SHEET								
HLA-A*24 Add-on SSP kit ²								
					Well			
					1	2	3	
					Prod. No.:	201781001	201781002	201781003
	IHC 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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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.

Additional primers in primer mix 3 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer.

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