

101.435-06 – including *Taq* polymerase, IFU-01
101.435-06u – without *Taq* polymerase, IFU-02

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“Instructions for Use” (IFU)

Lot No.: **54X**

Lot-specific information
Olerup SSP® HLA-A*69

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Product number:	101.435-06 – including <i>Taq</i> polymerase 101.435-06u – without <i>Taq</i> polymerase
Lot number:	54X
Expiry date:	2017-April-01
Number of tests:	6
Number of wells per test:	5+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. 54X.

Complete product documentation consists of generic Instructions for Use (IFU), lot specific Product Insert, Worksheet and Certificate.

The HLA-A*69 kit enables 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

Well 6 contains Negative Control primer pairs.

The kit design is based on IMGT/HLA database 3.17.0.

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.

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Well 6 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*69 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-A*69:01 to HLA-A*69:03 alleles.

PLATE LAYOUT

Each test consists of 6 PCR reactions in an 8 well cut PCR plate. Wells 7 to 8 are empty.

1	2	3	4	5	NC	empty	empty
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The 8 well cut PCR plate is marked with 'A69' in silver/gray ink.

Well No. 1 is marked with the Lot No. '54X'.

Wells 1 to 5 – HLA-A*69 high resolution primers.

Well 6 – 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, non-HLA-A*69 alleles will be amplified by all primer mixes.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*69 alleles, i.e. **A*69:01 to A*69:03**, recognized by the HLA Nomenclature Committee in July 2014^{1,2} will be amplified by the primers in the HLA-A*69 SSP kit.

The HLA-A*69 kit enables separation of the confirmed HLA-A*69 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-C*69 alleles is listed below.

The HLA-A*69 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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¹HLA-A alleles listed on the IMGT/HLA web page 2014-July-25, release 3.17.0,
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>.

ALLELE CONFIRMATION STATUS

Allele Status¹

*69:01	Confirmed
*69:02	Unconfirmed
*69:03	Unconfirmed

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2014-July-25, release 3.17.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-A*69 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

HLA-A*69 SSP subtyping

Specificities and sizes of the PCR products of the 5+1 primer mixes used for HLA-A*69 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*69 alleles ³	Other amplified HLA-Class I alleles ⁴
1	410 bp	800 bp	*69:01-69:03	*02:55
2⁵	100 bp	1070 bp	*69:01, 69:03	*02:34-02:35:03, 02:56:01-02:56:02, 02:62, 02:78, 02:103, 03:01:01:01-03:07, 03:09-03:28, 03:31, 03:33, 03:35, 03:37-03:40, 03:42-03:61, 03:64-03:87, 03:90-03:106, 03:109-03:151, 03:153-03:171, 03:173-03:175, 03:177-03:197N, 03:199-03:200Q, 11:12, 24:24, 29:01:01:01-29:18, 29:21-29:29, 29:31-29:33, 29:35-29:47, 29:49-29:65, 29:67-29:71, 30:01:01-30:01:10, 30:08, 30:11:01-30:11:02, 30:14L-30:20, 30:23-30:26, 30:30, 30:35-30:43, 30:48-30:49, 30:52-30:56, 30:58-30:60, 30:62-30:63, 30:65, 30:71-30:75, 30:78N-30:79, 30:81-30:83, 30:86-30:89, 31:89, 32:17, 34:09, 66:02, 66:12, 66:16, 68:01:01:01-68:01:17, 68:01:19-68:02:07, 68:06-68:14, 68:16-68:19, 68:21:01-68:30, 68:32-68:39, 68:41-68:71, 68:73-68:84, 68:86-68:89, 68:91-68:92, 68:94N-68:96, 68:98-68:108, 68:110-68:121, 74:13
3	295 bp	1070 bp	*69:01, 69:03	*02:01:01:01-02:01:15, 02:01:17-02:01:19, 02:01:21-02:01:70, 02:01:72-02:01:81, 02:01:83-02:01:86, 02:01:88-02:01:109, 02:01:111-02:01:112, 02:03:01-02:03:08, 02:06:01-02:06:17, 02:09, 02:11:01-02:13, 02:16, 02:19-02:22:02, 02:24:01-02:32N, 02:34-02:38, 02:40:01-02:46, 02:48-02:49, 02:51-02:52, 02:54-02:56:02, 02:59-02:62, 02:64, 02:66-02:72, 02:74:01-02:83N, 02:85-02:93, 02:95-02:97:02, 02:99, 02:101:01-02:101:02, 02:104-02:107, 02:109, 02:111, 02:113N-02:114, 02:116-02:121, 02:123-02:129, 02:131-02:134, 02:136-02:147, 02:149-02:151, 02:153:01-02:153:02, 02:158-02:171:02, 02:173-02:178, 02:181-02:185, 02:187-02:190, 02:192-02:193, 02:195-02:198, 02:200-02:206, 02:208, 02:210-02:211:02, 02:214-02:218, 02:220-02:223N, 02:225N-02:228, 02:230, 02:233-02:241, 02:243:01-02:243:02, 02:246-02:254, 02:256-02:260, 02:262-02:264, 02:266-02:267, 02:269-02:270, 02:272-02:281, 02:283-02:285, 02:287-02:299, 02:301N, 02:304-02:318, 02:321N-02:323, 02:325-02:333, 02:336, 02:339-02:343, 02:347-02:349, 02:351-02:358, 02:360-02:368, 02:370-02:372, 02:374-02:375, 02:377-02:397, 02:399-02:402, 02:404-02:407, 02:409-02:412, 02:414-02:420, 02:422-02:425, 02:428, 02:430-02:431, 02:434-02:436, 02:438-02:448, 02:454-02:471, 02:475-02:476N, 02:479-02:483, 02:485-02:488, 02:490N-02:491, 02:493-02:494, 02:497-02:498, 02:500Q-02:501N, 02:503-02:506N, 02:508-02:510, 02:512, 02:514N-02:516N, 02:518-02:525N, 02:528-02:530, 02:533-02:538, 03:95,

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				24:14, 29:22, 33:22, 68:105
4	295 bp	1070 bp	*69:02	*01:20, 01:66, 02:346, 02:427, 26:22, 66:09
5	225 bp	1070 bp	*69:03	*02:12-02:13, 02:19, 02:27, 02:37-02:38, 02:44, 02:54, 02:142, 02:226N, 02:280, 02:417, 24:14, 24:93
6⁶				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*69 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 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.

⁴Due to the sharing of sequence motifs between HLA-A alleles, non-HLA-A*69 alleles will be amplified by all primer mixes.

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

⁶Primer mix 6 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.

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5
Length of spec. PCR product	410	100	295	295	225
Length of int. pos. control ¹	800	1070	1070	1070	1070
5'-primer(s) ²	261	282	341	341	355
	5' -AAC 3'	5' -CAg 3'	5' -ggC 3'	5' -ggA 3'	5' -CCg 3'
3'-primer(s) ³	391	341	355	355	539
	5' -CCA 3'	5' -Cgg 3'	5' -gAC 3'	5' -gAC 3'	5' -TCT 3'
Well No.	1	2	3	4	5

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-A69 SSP kit ²								
				Well				
				1	2	3	4	5
				201432801	201432802	201432803	201432804	201432805
				Lot No.:				
	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	-	-	+	-	-

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.

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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 4 were available.

The specificities of the primers in primer solution 4 were tested by separately adding one 5'-primer, respectively one 3'-primer.

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