

Lot No.: **77F**

Lot-specific information

Olerup SSP[®] HLA-A*29

Product number: 101.428-12u – without *Taq* polymerase
Lot number: 77F
Expiry date: 2011-April-01
Number of tests: 12
Number of wells per test: 16
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. 77F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP[®]* HLA-A*29 LOT

The HLA-A*29 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP[®]* HLA-A*29 lot was made (**Lot No. 64E**).

The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot.

Well	5'-primer	3'-primer	rationale
6	Added	Added	Primer pair added for the A*2919 allele.
7	Added	Added	Primer pair added for the A*2919 allele.
12	New, moved	New, moved	New primer pair for the A*2918 allele, primer pair moved to well 13
13	Added	Added	Primer pair from well 12.

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PRODUCT DESCRIPTION

HLA-A*29 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*2901 to A*2919 alleles.

PLATE LAYOUT

Each test consists of 16 PCR reactions in a 16 well cut PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16

The 16 well cut PCR plate is marked with 'HLA-A*29' in silver/gray ink.

Well No. 1 is marked with the Lot No. '77F'.

The PCR plates are covered with a PCR-compatible foil.

Please note: When removing each 16 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

The interpretation of HLA-A*29 SSP subtypings will be influenced by the A*0327, the A*230301, three A*24, six A*31, most A*32, the A*3310 and the A*74 alleles when present on the other haplotype.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*29 alleles, i.e. **A*2901 to A*2919 alleles**, recognized by the HLA Nomenclature Committee in January 2009¹ will give rise to unique amplification patterns by the primers in the HLA-A*29 subtyping kit².

The HLA-A*29 subtyping kit cannot distinguish the A*29010101 and A*290102 and the A*290201 to A*290203 alleles.

¹HLA-A alleles listed on the IMGT/HLA web page 2009-January-16, release 2.24.0, www.ebi.ac.uk/imgt/hla.

²The A*2918 will give rise to identical amplification pattern as most A*32 and A*74 alleles with the HLA-A*29 subtyping kit. The A*2918 allele can be distinguished from the A*32 and A*74 alleles by the HLA-A low resolution kit.

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RESOLUTION IN HOMO- AND HETEROZYGOTES

The 20 different amplification patterns generated by the 19 HLA-A*29 alleles can be combined in 210 homozygous and heterozygous combinations. Fifty-four of these genotypes do not give rise to unique amplification patterns. The different sizes of the specific PCR products generated by primer mixes 6, 7, 9 and 10 were not considered in these calculations.

++-----	-----	2901,29010102N = 29010102N,29010102N
+-------	++-----	2908N,2917 = 2909,2916
+-------	+---+---	2908N,2912 = 2911,2916
+-------	+-----	2901,2908N = 2902,2916 = 2908N,2916
+-------	-+---+---	2909,2912 = 2911,2917
+-------	-+-----	2901,2909 = 2902,2917 = 2909,2917
+-------	-----+---	2901,2911 = 2902,2912 = 2911,2912
+-----	+-----	2901,2916 = 2916,2916
+-----	-+-----	2901,2917 = 2917,2917
+-----	-----+---	2901,2912 = 2912,2912
+-----	-----++	2901,2915 = 2915,2915
--++-----	-----	2902,2903 = 2903,2903
--+-----	-----	2902,2904 = 2904,2904
--+-----	-----	2902,2919 = 2905,2906 = 2905,2919 =
		2906,2919 = 2919,2919
--+-----	-----	2902,2905 = 2905,2905
--+-----	-----	2902,2906 = 2906,2906
--+-----	-----	2902,2907 = 2907,2907
--+-----	+-----	2902,2908N = 2908N,2908N
--+-----	-+-----	2902,2909 = 2909,2909
--+-----	--+-----	2902,2910 = 2910,2910
--+-----	-----+---	2902,2911 = 2911,2911
--+-----	-----+---	2902,2913 = 2913,2913
--+-----	-----+---	2902,2914 = 2914,2914

2901 = 29010101 and 290102

2902 = 290201-290203

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SPECIFICITY TABLE

HLA-A*29 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-A*29 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*29 alleles	Other amplified HLA-A alleles ³
1	480 bp	800 bp	29010101-29010102N, 2912, 2915-2917	
2⁴	130 bp	1070 bp	29010102N	
3	440 bp	800 bp	290201-2911, 2913, 2914, 2919	
4	165 bp	800 bp	2903	230301, 3105, 3213, 3310
5⁴	130 bp	1070 bp	2904	
6^{4,8}	105, 130 bp	800 bp	2905, 2919	3124, 3202
7^{4,5,9}	105, 210 bp	1070 bp	2906, 2919	3212
8⁴	85 bp	1070 bp	2907	2417, 2441
9^{4,6,10}	80, 170 bp	1070 bp	2908N, 2916	0327
10^{4,11}	95, 170 bp	800 bp	2909, 2917	3124
11	195 bp	1070 bp	2910	
12^{4,7}	80 bp	1070 bp	2918	32010101-3203, 3205-3218, 7401-7413
13^{4,6,12}	85, 165 bp	1070 bp	2911, 2912	3116
14	260 bp	1070 bp	2913	2482, 3107, 3108, 3110
15⁴	100 bp	1070 bp	2914	
16⁴	95 bp	1070 bp	2915	

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*29 SSP typings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective lengths of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

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

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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 two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-A*29 subtyping.

In addition, wells number 3, 4, 6 and 10 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

In the presence of a specific amplification the intensity of the control band often decreases.

³Due to the sharing of sequence motifs between HLA-A alleles a few non-HLA-A*29 alleles will be amplified by primer mixes 4, 6 to 10, and 12 to 14.

The A*2918 allele will give rise to identical amplification pattern as most A*32 and A*74 alleles with the HLA-A*29 subtyping kit. The A*2918 allele can be distinguished from A*32 and A*74 alleles by the HLA-A low resolution kit.

⁴Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁵Primer mixes 7 may have a tendency of giving rise to a primer oligomer artefact.

⁶Primer mixes 9 and 13 may give rise to nonspecific amplifications.

⁷Primer mix 12 may give a lower yield of specific PCR product than the other A*29 primer mixes.

⁸Primer mix 6: Specific PCR fragment of 105 bp in the A*2919 allele. Specific PCR fragment of 130 bp in the A*2905 and in the A*3124 and 3202 alleles.

⁹Primer mix 7: Specific PCR fragment of 105 bp in the A*2919 allele. Specific PCR fragment of 210 bp in the A*2906 and in the A3212 alleles.

¹⁰Primer mix 9: Specific PCR fragment of 90 bp in the A*2916 and in the A*0327 alleles. Specific PCR fragment of 170 bp in the A*2908N allele.

¹¹Primer mix 10: Specific PCR fragment of 95 bp in the A*2909 and in the A*3124 alleles. Specific PCR fragment of 170 bp in the A*2917 allele.

¹²Primer mix 13: Specific PCR fragment of 85 bp in the A*2911 allele. Specific PCR fragment of 165 bp in the A*2912 and in the A*3116 alleles.

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INTERPRETATION TABLE								
HLA-A*29 SSP subtyping								
Amplification patterns of the A*2901 to 2919 alleles								
	Well⁴							
	1	2	3	4	5	6	7	8
Length of spec.	480	130	440	165	130	105	105	85
PCR product						130	210	
Length of int.	800	1070	800	800	1070	800	1070	1070
pos. control¹								
5'-primer(s)²	180	808	219	448	180	219	219	368
	5'-TTT ^{3'}	5'-CgT ^{3'}	5'-gCA ^{3'}	5'-CCT ^{3'}	5'-TTT ^{3'}	5'-gCA ^{3'}	5'-gCA ^{3'}	5'-gTT ^{3'}
						448	448	
						5'-CCT ^{3'}	5'-CCT ^{3'}	
3'-primer(s)³	376	895	376	570	268	282	282	413
	5'-gTg ^{3'}	5'-CTC ^{3'}	5'-gTC ^{3'}	5'-CCg ^{3'}	5'-ATg ^{3'}	5'-gAg ^{3'}	5'-gAg ^{3'}	5'-gCC ^{3'}
						539	616	
						5'-TCT ^{3'}	5'-CgC ^{3'}	
Well No.	1	2	3	4	5	6	7	8
HLA-A allele								
*29010101, 290102	1							
*29010102N	1	2						
*290201-290203			3					
*2903			3	4				
*2904			3		5			
*2905			3			6		
*2906			3				7	
*2907			3					8
*2908N			3					
*2909			3					
*2910			3					
*2911			3					
*2912	1							
*2913			3					
*2914			3					
*2915	1							
*2916	1							
*2917	1							
*2918, 320101-320103, 3203, 3205-3211Q, 3214- 3218, 7401-7413⁵								
*2919			3			6	7	
Well No.	1	2	3	4	5	6	7	8

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INTERPRETATION TABLE								
HLA-A*29 SSP subtyping								
Amplification patterns of the A*2901 to 2919 alleles								
Well⁴								
9	10	11	12	13	14	15	16	
80	95	195	80	85	260	100	95	Length of spec. PCR product
170	170			165				
1070	800	1070	1070	1070	1070	1070	1070	Length of int. pos. control ¹
97	130	448	180	97	98	180	484	5'-primer(s) ²
5'-TCA ^{3'}	5'-AgA ^{3'}	5'-CCT ^{3'}	5'-TTT ^{3'}	5'-TCA ^{3'}	5'-CAC ^{3'}	5'-TTT ^{3'}	5'-ACg ^{3'}	
413	448			448				
5'-CC6 ^{3'}	5'-CCT ^{3'}			5'-CCT ^{3'}				
224	257	601	218	221	317	238	538	3'-primer(s) ³
5'-TCT ^{3'}	5'-gCA ^{3'}	5'-CTT ^{3'}	5'-gCg ^{3'}	5'-ACA ^{3'}	5'-ggA ^{3'}	5'-CCT ^{3'}	5'-CAA ^{3'}	
454	502			494				
5'-CTg ^{3'}	5'-CTT ^{3'}			5'-TCg ^{3'}				
9	10	11	12	13	14	15	16	Well No.
								HLA-A allele
								*29010101, 290102
								*29010102N
								*290201-290203
								*2903
								*2904
								*2905
								*2906
								*2907
9								*2908N
	10							*2909
		11						*2910
				13				*2911
				13				*2912
					14			*2913
						15		*2914
							16	*2915
9								*2916
	10							*2917
			12					*2918, 320101-320103, 3203, 3205-3211Q, 3214- 3218, 7401-7413 ⁵
								*2919
9	10	11	12	13	14	15	16	Well No.

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Length of spec.	480	130	440	165	130	105	105	85
PCR product						130	210	
Well No.	1	2	3	4	5	6	7	8
*0327								
*230301, 3105, 3310				4				
*2417, 2441								8
*2482, 3107, 3108, 3110								
*3116								
*3124						6		
*3202						6		
*3212							7	
*3213				4				
HLA-A allele								
Well No.	1	2	3	4	5	6	7	8

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-A*29 subtyping. .

In addition, wells number 3, 4, 6 and 10 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

²The nucleotide position, in the 2nd, 3rd or 4th exons, 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, in the 2nd, 3rd or 4th exons, 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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80	95	195	80	85	260	100	95	Length of spec. PCR product
9	10	11	12	13	14	15	16	Well No.
9								*0327
								*230301, 3105, 3310
								*2417, 2441
				14				*2482, 3107, 3108, 3110
				13				*3116
	10							*3124
			12					*3202
			12					*3212
			12					*3213
								HLA-A allele
9	10	11	12	13	14	15	16	Well No.

⁴Primer mix 6: Specific PCR fragment of 105 bp in the A*2919 allele. Specific PCR fragment of 130 bp in the A*2905 and in the A*3124 and 3202 alleles.

Primer mix 7: Specific PCR fragment of 105 bp in the A*2919 allele. Specific PCR fragment of 210 bp in the A*2906 and in the A3212 alleles.

Primer mix 9: Specific PCR fragment of 90 bp in the A*2916 and in the A*0327 alleles. Specific PCR fragment of 170 bp in the A*2908N allele.

Primer mix 10: Specific PCR fragment of 95 bp in the A*2909 and in the A*3124 alleles. Specific PCR fragment of 170 bp in the A*2917 allele.

Primer mix 13: Specific PCR fragment of 85 bp in the A*2911 allele. Specific PCR fragment of 165 bp in the A*2912 and in the A*3116 alleles.

⁵The A*2918 will give rise to identical amplification pattern as most A*32 and A*74 alleles with the HLA-A*29 subtyping kit. The A*2918 allele can be distinguished from A*32 and A*74 alleles by the HLA-A low resolution kit.

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CELL LINE VALIDATION SHEET																				
HLA-A*29 SSP subtyping kit																				
				Well																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Lot No.:	200958401	200958402	200958403	200958404	200958405	200958406	200958407	200958408	200958409	200958410	200958411	200958412	200958413	200958414	200958415	200958416
	IHWC cell line	A*	A*																	
1	9001 SA	*2402		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707	*0201		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324	*0101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373	*3001		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*0101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM	*0201	*2603	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL	*2601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9007 DEM	*0201		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*2601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3	*2402		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT	*2902		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*0201		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM	*0201		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*3101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*2402		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*2902		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540	*0301	*8001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*3303	*7401	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
19	9038 BM16	*0201		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*0201		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*0217		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*0201		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*0201	*3401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*3201		-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
25	9049 IBW9	*3301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*0205		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*2410	*2901	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB	*0201	*2902	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*2902		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*3001	*6802	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*3002		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*0201		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*3101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*3101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*3201		-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
36	9024 KT17	*0206	*1101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB	*0301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*0217		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML	*0101	*0301	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*0207	*3001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*0301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*0207		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*0207		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*6601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*2301	*2402	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU	*0301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*0216	*0301	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND	*0201	*1101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-A*29 SSP

Product number: 101.428-12u – without *Taq* polymerase
Lot number: 77F
Expiry date: 2011-April-01
Number of tests: 12
Number of wells per test: 16

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2009-584-01	9	2009-584-09
2	2009-584-02	10	2009-584-10
3	2009-584-03	11	2009-584-11
4	2009-584-04	12	2009-584-12
5	2009-584-05	13	2009-584-13
6	2009-584-06	14	2009-584-14
7	2009-584-07	15	2009-584-15
8	2009-584-08	16	2009-584-16

The specificity of each primer solution of the kit has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 2, 4 to 7, 9 to 11 and 13 to 16 were available. The specificities of the primers in primer solutions 4, 6, 7, 10, 11, 14 and 15 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 2, 5, 9 and 13 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solution 16 it was only possible to the 3'-primer, the 5'-primer was not possible to test. In primer solution 7 one of the 3'-primers was not possible to test, and in primer solution 10 one of the 5'-primers was not possible to test.

Results: No false positive or false negative amplifications were obtained.

Date of approval: 2009-May-25

Approved by:

Quality Control, Supervisor

Lot No.: **77F**

Lot-specific information

Declaration of Conformity

Product name: *Olerup* SSP® HLA-A*29
Product number: 101.428-12u
Lot number: 77F

Intended use: HLA-A*29 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Hasselstigen 1
SE-133 33 Saltsjöbaden, Sweden
Phone: +46-8-717 88 27
Fax: +46-8-717 88 18

We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2000 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, conformity assessed using Annex IV, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom. (Notified Body number: 0088.)

Saltsjöbaden, Sweden
2009-May-25

Olle Olerup
Managing Director

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