Olerup SSP® HLA-A low resolution

Product number:	**101.401-48/12 – including *Taq* polymerase**
Lot number:	**94M**
Expiry date:	**2014-May-01**
Number of tests:	**48 tests – Product No. 101.401-48**
	12 tests – Product No. 101.401-12
Number of wells per test:	**23 +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. 94M.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® HLA-A LOW RESOLUTION LOT

Lot No. 94M of the HLA-A low resolution kit is manufactured using white plastic trays.

The **HLA-A low resolution** specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup* SSP® HLA-A low resolution lot was made **(Lot No. 04L)**.

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

Well	5'-primer	3'-primer	rationale
2	Added	-	Improved yield of specific PCR product.
8	-	Added	Primer added for the A*25:15 allele.
9	Added	-	Primer added for the A*25:15 allele.
12	Added	Added	Primer added for improved yield of the HLA-specific product for the A*34:02 motif.
13	-	Modified	Improved yield of specific PCR product.
15	Added	-	Primers added for the A*30:47 and 30:52 alleles.

Change in revision R01 compared to R00:

1. The HLA-A*68:13 allele is amplified by primer mix 4. This has been corrected in the specificity and interpretation tables.

Change in revision R03 compared to R02:

1. The HLA-A*03:01:03, 03:09, 03:23:01, 03:89, 03:108, 11:06, 11:18, 24:28, 24:89, 26:03:01-26:03:02, 26:06, 26:07:02, 26:21, 26:30, 30:13, 30:16, 30:44, 30:46, 68:05, 68:15, 68:20 and 68:30 alleles are weakly amplified by primer mix 2. This has been corrected in the Specificity and Interpretation Tables.

Changes in revision R04 compared to R03:

1. In primer mix 5, the specific PCR product of 535 base pairs may be difficult to distinguish from the internal control band. A foot note has been added to the Specificity Table.
2. Primer mix 17 does not amplify the HLA-A*36:02 allele. This has been corrected in the Specificity and Interpretation Tables.

Well 24 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 the amplicons generated by control primer pairs.

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 product	**105**	**200**	**105**	**80**	**75**	**80**
5'-primer[1]	**164**	**340**	**440**	**45**	**45**	**43**
	5'-CAC3'	**5'-Agg3'**	**5'-TTA3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-Tgg3'**
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**
A*	**+**	**+**	**+**			
B*	**+**	**+**	**+**			
C*	**+**	**+**	**+**			
DRB1				**+**	**+**	
DRB3				**+**	**+**	
DRB5				**+**		
DQB1					**+**	
DPB1						**+**

[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.

PRODUCT DESCRIPTION

HLA-A low resolution

CONTENT

The primer set contains 5'- and 3'-primers for grouping the HLA-A*01:01 to A*80:02 alleles into the corresponding serological groups A1 to A80.

PLATE LAYOUT

Each test consists of 24 PCR reactions in a 24 well PCR plate. This lot is manufactured using white plastic trays.

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
9	**10**	**11**	**12**	**13**	**14**	**15**	**16**
17	**18**	**19**	**20**	**21**	**22**	**23**	**24**

Wells 1 to 23 – HLA-A low resolution primers.
Well 24 – Negative Control.
The 24 well PCR plate is marked with 'HLA-A low' in silver/gray ink.
Well No. 1 is marked with the Lot No. '94M'.
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 covered with a PCR-compatible foil.
Please note: When removing each 24 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

Only HLA-A alleles will be amplified by the 23 wells of the HLA-A low resolution primer set except that primer mix 6 will amplify the B*18:27 allele. Thus, the interpretation of HLA-A low resolution is only influenced by this HLA-B allele and not by other HLA Class I genes.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A alleles, i.e. **A*01:01 to A*80:02**, recognized by the HLA Nomenclature Committee in July 2011[1] will be amplified by the primers in the HLA-A low resolution primer set. The HLA-A alleles will be grouped into their corresponding serological specificities[2].

[1]HLA-A, HLA-B and HLA-C alleles listed on the IMGT/HLA web page 2011-July-14, release 3.5.0, www.ebi.ac.uk/imgt/hla.
[2]The A*23:14 and the A*24:05, 24:13:02 and 24:24 alleles will give rise to identical amplification patterns. These four alleles can be separated by the respective high resolution SSP primer sets.

SPECIFICITY TABLE

HLA-A low resolution primer set

Specificities and sizes of the PCR products of the 23+1 primer mixes used for HLA-A low resolution SSP typing

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	HLA-A serology[3]	Amplified HLA-A alleles[4,5,6]
1[7,8]	120 bp, 145 bp, 225 bp	**800 bp**	A1, A36	*01:01:01:01-01:04N, 01:06-01:33, 01:35-01:101, 03:18, 11:94, 36:01-36:05
2[8]	210 bp, 255 bp, 365 bp, 415 bp	**800 bp**	A2,A19, A28, A203, A210, A3, A11, A9, A26, A30, A68	*02:01:01:01-02:01:15, 02:01:17-02:01:19, 02:01:21-02:22:02, 02:24:01-02:35:01, 02:35:03-02:47, 02:49-02:77, 02:78[w], 02:79:01-02:97:02, 02:99, 02:101:01-02:128, 02:130-02:323, 02:325-02:326, 03:01:03[w], 03:09[w], 03:23:01[w], 03:89[w], 03:108[w], 11:06[w], 11:18[w], 24:28[w], 24:89[w], 26:03:01-26:03:02[w], 26:06[w], 26:07:02[w], 26:21[w], 26:30[w], 30:13[w], 30:16[w], 30:44[w], 30:46[w], 68:05[w], 68:15[w], 68:20[w], 68:30[w]
3[9]	205 bp, 235 bp	1070 bp	A1, A3, A11, A32, A34, A36	*01:12, 01:19, 01:21, 03:01:01:01-03:17, 03:19-03:74, 03:76-03:94, 03:96-03:132, 11:25, 11:60, 24:92, 32:04, 33:49[w], 34:02:01, 34:02:02[w], 34:03-34:04, 34:07-34:09, 36:02
4	190 bp	**800 bp**	A1, A2, A3, A11, A26, A30, A36, A68	*01:01:01:01-01:01:22, 01:01:24-01:04N, 01:06-01:07, 01:09-01:11N, 01:13, 01:16N-01:18N, 01:20-01:29, 01:31N-01:33, 01:35-01:78, 01:80-01:98, 01:100-01:101, 02:78, 02:169, 03:12, 03:18, 03:88, 11:01:01-11:27, 11:29-11:106, 26:19, 30:08, 36:04, 68:13, 68:66
5[14]	160 bp, 535 bp	**800 bp**	A3,A9,A23, A24, A31, A32	*01:95, 03:30, 23:01:01-23:46, 24:02:01:01-24:11N, 24:13:01-24:15, 24:17-24:64, 24:66-24:88, 24:90N-24:128, 24:130-24:182, 31:08, 32:05, 32:13
6[11]	135 bp, 175 bp, 210 bp	**800 bp**	A9,A23, A24, A29, A80	*23:01:01-23:46, 24:05, 24:13:02, 24:24, 29:07, 31:29, 80:01-80:02, **B*18:27**

7	175 bp, 205 bp	1070 bp	A2, A23, A24, A26, A33	*02:17:01^{W}-02:17:02^{W}, 23:14, 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, 24:139-24:182, 26:16, 33:19, 68:45
8	165 bp, 200 bp	**800 bp**	A2, A3, A10, A11, A25, A26, A28,A32, A34, A66, A68, A69	*01:51, 02:55, 03:24, 03:50, 11:10, 25:01:01-25:16, 26:01:01-26:06, 26:08-26:15, 26:17-26:18, 26:20-26:43:02, 26:45-26:63, 26:65-26:69, 29:28, 32:15, 33:51, 34:01:01-34:09, 66:01-66:16, 68:01:01:01-68:83, 69:01
9[7]	75 bp	**800 bp**	A25, A32	*25:01:01-25:16, 32:01:01-32:02, 32:04, 32:06-32:36
10[7]	85 bp	1070 bp	A2, A10, A26, A33	*01:51, 01:83, 02:146, 26:01:01-26:02, 26:04, 26:07:01-26:18, 26:20, 26:22-26:29, 26:31-26:43:02, 26:45-26:69, 33:13, 33:48
11[7,8]	80 bp, 175 bp, 500 bp	1070 bp	A1, A11, A24, A26, A31, A34, A66	*01:13, 01:28, 03:63, 03:88, 11:01:01-11:27, 11:29-11:106, 24:19, 24:44, 26:03:01-26:03:02, 26:06, 26:21, 31:03, 34:01:01-34:08, 66:01, 66:04-66:11, 66:13-66:15, 80:02
12[7]	125 bp, 185 bp	**800 bp**	A11, A25, A26, A31, A34, A43, A66	*02:309, 03:01:19, 11:11, 25:05-25:06, 26:09, 26:54, 31:03-31:04, 34:01:01-34:09, 43:01, 66:02-66:03, 66:16
13	175 bp, 225 bp	1070 bp	A1, A2, A3, A25, A26, A34, A43, A66, A74	*01:13, 02:34-02:35:03, 02:56:01-02:56:02, 02:62, 02:103, 02:135, 03:01:01:01-03:01:22, 03:01:24-03:07, 03:09-03:11N, 03:13-03:31, 03:33-03:35, 03:37-03:40, 03:42-03:56, 03:58, 03:60-03:71, 03:73-03:87, 03:90-03:106, 03:109-03:110, 03:112-03:132, 25:01:01-25:05, 25:07-25:16, 26:01:01-26:01:20, 26:01:22, 26:02^{W}, 26:03:01-26:03:02, 26:05-26:08, 26:10-26:33, 26:35-26:43:02, 26:45-26:69, 30:55, 34:08, 43:01, 66:01, 66:04-66:15, 68:71, 74:13
14[7]	100 bp, 200 bp, 240 bp	1070 bp	A29, A33	*02:237, 02:309, 03:95, 26:19, 26:22, 29:01:01:01-29:31, 31:03-31:04, 33:13, 33:48, 34:04, 66:09
15[7,8,10,12]	90 bp, 135 bp, 205 bp	1070 bp	A1, A30	*01:13, 01:28, 03:43, 03:82, 30:01:01-30:04:02, 30:06-30:20, 30:22-30:55, 31:35

16	240 bp, 370 bp, 395 bp	1070 bp	A31, A32	*02:237, 03:95, 29:14, 31:01:02-31:54, 32:05
17	140 bp, 180 bp	1070 bp	A29, A32	*01:95, 03:43, 03:82, 29:13, 31:35, 32:01:01-32:03, 32:05-32:36, 36:02, 74:07
18	200 bp	1070 bp	A33, A68	*02:243, 33:01:01-33:01:06, 33:03:01-33:50, 68:29
19	160 bp, 200 bp	**800 bp**	A74	*29:19, 74:01-74:15
20[11]	220 bp, 245 bp	**800 bp**	A2, A210, A25, A68	*02:34-02:35:03, 02:46, 02:48, 02:56:01-02:56:02, 02:62, 02:70, 02:78, 02:103, 02:129, 25:05, 26:54, 68:01:01:01-68:83
21	240 bp, 375 bp	**800 bp**	A2, A24, A26, A68, A69	*02:55, 02:243, 24:82, 26:22, 33:22, 66:09, 68:29, 69:01
22[7,13]	85 bp, 240 bp	**800 bp**	A2, A36	*02:34-02:35:03, 02:46, 02:48, 02:56:01-02:56:02, 02:62, 02:70, 02:78, 02:103, 02:129, 36:01-36:05
23[7,11]	75 bp, 160 bp, 240 bp, 495 bp	**800 bp**	A2, A26, A68, A80	*02:55, 03:41, 03:63, 03:75, 03:88, 24:18, 26:03:01-26:03:02, 26:05-26:06, 26:21, 26:30, 33:24, 36:02, 68:05, 68:15, 68:20, 80:01
24[15]	**-**	**-**	**-**	**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 low resolution 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 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 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 low resolution typing.
In addition, wells number 2, 4 to 6, 8, 9, 12 and 19 to 23 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.
[3]The serological reactivity of all HLA-A alleles is not known. In this table we use the expert-assigned serological grouping in Tissue Antigens (2009) 73:95-170 and the serological grouping of the sequence-defined allele.
[4]For several HLA-A alleles only partial 1st exon nucleotide sequences are available. We assume that unknown sequences are conserved within allelic groups.
Nucleotide sequence information is available for only exons 2 and 3 of many HLA Class I alleles and for only exon 2 of many HLA Class II alleles and not for other exons or for the introns of these alleles. We assume that unknown sequences in these exons and in the introns are conserved within loci and within allelic groups.
[5]The A*23:14 and the A*24:05, 24:13:02 and 24:24 alleles will give rise to identical amplification patterns. These four alleles can be separated by the respective high resolution SSP primer sets.
[6]Primer mix 6 will amplify the B*18:27 allele.
[7]Specific PCR fragments shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR bands.
[8]The primer pairs in wells 1, 2, 11 and 15 will in many samples give rise to two or three HLA-specific PCR fragments.
[9]Primer mix 3 may faintly amplify the A*30:04, 30:06, 30:17 and 30:29 alleles.
[10]Primer mix 15 may yield less specific PCR product than the other primer mixes.
[11]Primer mixes 6, 20 and 23 may have a tendency of primer dimer formation.
[12]Primer mix 15 may have tendencies of unspecific amplifications.
[13]Primer mix 22 might faintly amplify most A*11 alleles.
[14]In primer mix 5, the specific PCR product of 535 base pairs may be difficult to distinguish from the internal control band. The alleles giving rise to a product of this size are the following:
A*01:95, 23:09, 24:02:06, 24:02:27, 24:08, 24:24, 24:29 , 24:42, 24:67, 24:116, 24:137, 24:140, 24:145, 24:156, 24:171 and 32:13.
[15]Primer mix 32 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.

INTERPRETATION TABLE

HLA-A low resolution SSP typing

Amplification patterns of the A*01:01 to A*80:02 alleles

	Well[6,8]																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Length of spec.	120	210	205	190	160	135	175	165	75	85	80	125	175	100	90	240	140	200	160	220	240	85	75	Neg. Control
PCR product(s)	145	255	235		535	175	205	200			175	185	225	200	135	370	180		200	245	375	240	160	
	225	365				210					500			240	205	395							240	
		415																					495	
Length of int. pos. control[1]	800	800	1070	800	800	800	1070	800	800	1070	1070	800	1070	1070	1070	1070	1070	1070	800	800	800	800	800	
5'-primer(s)[2]	98	48	363	98	144	176	98	98	266	257	301	103	98	98	203	41	180	98	180	78	28	78	176	Negative Control
	5'-CTT 3'	5'-gCT 3'	5'-ATA 3'	5'-CTA 3'	5'-gCC 3'	5'-gCA 3'	5'-CTC 3'	5'-CTA 3'	5'-ACg 3'	5'-Cgg 3'	5'-Cgg 3'	5'-CCT 3'	5'-CTT 3'	5'-CAC 3'	5'-gAA 3'	5'-CTT 3'	5'-TTT 3'	5'-CAC 3'	5'-TTT 3'	5'-TCT 3'	5'-TCg 3'	5'-TCT 3'	5'-gCA 3'	
	103	78		413	317	368	368	102	266	259	302	423	423	238	362	355	203			106	261	527	261	
	5'-CCT 3'	5'-TCT 3'		5'-CCg 3'	5'-gCT 3'	5'-gTT 3'	5'-gTT 3'	5'-ACA 3'	5'-ACg 3'	5'-CgA 3'	5'-ggA 3'	5'-gCT 3'	5'-gCT 3'	5'-AgA 3'	5'-ggT 3'	5'-CCg 3'	5'-gAA 3'			5'-CCA 3'	5'-AAC 3'	5'-TgC 3'	5'-AAC 3'	
	123	106						413	266	261	385			355	363		418			2nd			341	
	5'-AgT 3'	5'-CCA 3'						5'-CCg 3'	5'-Agg 3'	5'-AAC 3'	5'-ggC 3'			5'-CCg 3'	5'-ATA 3'		5'-AgC 3'			5'-CCT 3'			5'-ggA 3'	
	363																						355	
	5'-ATA 3'														5'-ATA 3'	363						5'-CCC 3'	
															369									
															5'-TAC 3'									
3'-primer(s)[3]	203	240	527	256	265	270	259	259	302	299	341	257	282	257	299	238	290	256	299	265	97	265	292	
	5'-TCT 3'	5'-ggA 3'	5'-CCA 3'	5'-CTg 3'	5'-CCC 3'	5'-ACA 3'	5'-gTT 3'	5'-gTT 3'	5'-ggC 3'	5'-TCg 3'	5'-CgT 3'	5'-gCA 3'	5'-gAC 3'	5'-gCA 3'	5'-CCA 3'	5'-CCT 3'	5'-CAA 3'	5'-CCC 3'	5'-CCA 3'	5'-CCC 3'	5'-ggT 3'	5'-CCC 3'	5'-gTg 3'	
	545	292	527	559	570	502	502	259			521	506	282	299	411	243	317	256	299	282	355	282	292	
	5'-AgA 3'	5'-gTg 3'	5'-CCT 3'	5'-CCg 3'	5'-CCg 3'	5'-CTg 3'	5'-CTT 3'	5'-gTT 3'			5'-ggg 3'	5'-TgT 3'	5'-gAC 3'	5'-TCg 3'	5'-TCA 3'	5'-TCA 3'	5'-ggA 3'	5'-CTC 3'	5'-CCA 3'	5'-gAC 3'	5'-gAC 3'	5'-gAC 3'	5'-gTT 3'	
			555			538	539	538				559	559	418	526	265	555	259	341	282		282	299	
			5'-gCA 3'			5'-CAg 3'	5'-TCT 3'	5'-CCA 3'				5'-CTC 3'	5'-CCg 3'	5'-gTC 3'	5'-CCA 3'	5'-CCC 3'	5'-CCA 3'	5'-gTT 3'	5'-CgT 3'	5'-gAC 3'		5'-gAC 3'	5'-TCT 3'	
			555									559		555		555				502		570	555	
			5'-CCA 3'									5'-CgT 3'		5'-CCA 3'		5'-CCA 3'				5'-CTT 3'		5'-CAC 3'	5'-CCA 3'	
																				506				
																			5'-TgT 3'					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
HLA-A allele[4]	ser[5]																								
*01:01:01:01-01:01:22, 01:01:24-01:04N, 01:06-01:07, 01:09-01:11N, 01:16N-01:18N, 01:20, 01:22N-01:27N, 01:29, 01:31N-01:33, 01:35-01:50, 01:52N-01:78, 01:80-01:82, 01:84-01:94, 01:96-01:98, 01:100-01:101	A1, Null, –	1			4																				Negative Control
*01:01:23, 01:08, 01:14-01:15N, 01:30, 01:79, 01:99	A1, Null, –	1																							
*01:12, 01:19	A1, –	1		3																					
*01:13	A1	1			4							11		13		15									
*01:21	A1	1		3	4																				
*01:28	–	1			4							11				15									
*01:51	–	1			4				8		10														
*01:83	–	1			4						10														
*01:95	–	1			4	5												17							
*02:01:01:01-02:01:15, 02:01:17-02:01:19, 02:01:21-02:16, 02:18-02:22:02, 02:24:01-02:33, 02:36-02:45, 02:47, 02:49-02:54, 02:57-02:61, 02:63-02:69, 02:71-02:77, 02:79:01-02:97:02, 02:99, 02:101:01-02:102, 02:104-02:128, 02:130-02:134, 02:136-02:145, 02:147-02:168, 02:170-02:236, 02:238-02:242, 02:244-02:308, 02:310-02:323, 02:325-02:326	A2, A19, Low A2, A203, A210, Null, –		2																						
*02:17:01-02:17:02	A2		2					w																	
*02:34-02:35:01, 02:35:03, 02:56:01-02:56:02, 02:62, 02:103	A2		2											13							20		22		
*02:35:02	A2													13							20		22		
*02:46, 02:70	A2		2																		20		22		
*02:48, 02:129	A2, –																				20		22		
*02:55	A2, A28		2						8													21		23	
Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
HLA-A allele[4]	ser[5]																								Negative Control
*02:78	–		w		4																20		22		
*02:135	–		2											13											
*02:146	–		2								10														
*02:169	–		2		4																				
*02:237	–		2												14		16								
*02:243	–		2																18			21			
*02:309	–		2										12		14										
*03:01:01:01-03:01:02, 03:01:04-03:01:18, 03:01:20-03:01:22, 03:01:24-03:07, 03:10-03:11N, 03:13-03:17, 03:19-03:22:02, 03:23:02, 03:25-03:29, 03:31, 03:33-03:35, 03:37-03:40, 03:42, 03:44-03:49, 03:51-03:56, 03:58, 03:60-03:62, 03:64-03:71, 03:73-03:74, 03:76-03:81, 03:83-03:87, 03:90-03:94, 03:96-03:106, 03:109-03:110, 03:112-03:132	A3, Null, –			3										13											
*03:01:03, 03:09, 03:23:01	A3, –		w	3										13											
*03:01:19	–			3									12	13											
*03:01:23, 03:08, 03:32, 03:36N, 03:57, 03:59, 03:72, 03:107, 03:111	A3, Null, –			3																					
*03:12	A3			3	4																				
*03:18	–	1			4									13											
*03:24, 03:50	A3, –			3					8					13											
*03:30	A3			3		5								13											
*03:41	–			3																				23	
*03:43, 03:82	–			3										13		15		17							
*03:63	–			3								11		13										23	
*03:75	–													13										23	
*03:88	–			3	4							11												23	
*03:89,03:108	–		w	3																					
*03:95	–													13	14		16								
*11:01:01-11:05, 11:07-11:09, 11:12-11:17, 11:19-11:24:02, 11:26-11:27, 11:29-11:59, 11:61-11:93, 11:95-11:106	A11, Null, –				4							11													
Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
HLA-A allele[4]	ser[5]																								
*11:06, 11:18	A11, –		w		4							11													Negative Control
*11:10	A11				4				8			11													
*11:11	–				4							11	12												
*11:25, 11:60	A11, –			3	4							11													
*11:94	–	1			4							11													
*23:01:01-23:13, 23:15-23:46	A23, Null, –					5	6																		
*23:14, 24:05, 24:13:02, 24:24[7]	A23, A9, A24					5	6	7																	
*24:02:01:01-24:04, 24:06-24:11N, 24:13:01, 24:17, 24:20-24:23, 24:25-24:43, 24:45N-24:50, 24:54-24:56, 24:58-24:63, 24:66-24:81, 24:83N-24:88, 24:90N-24:91, 24:93, 24:95-24:113, 24:115-24:128, 24:130-24:137, 24:139-24:182	A24, Low A24, A2403, A9, Null, –					5		7																	
*24:14-24:15, 24:51-24:53, 24:57, 24:64, 24:94, 24:114, 24:138	A24, –					5																			
*24:18	A24, A3					5		7																23	
*24:19, 24:44	A9					5		7				11													
*24:28	A9		w			5		7																	
*24:82	–					5		7														21			
*24:89	–		w					7																	
*24:92	–			3		5																			
*24:129	–							7																	
*25:01:01-25:04, 25:07-25:16	A25, Null, –								8	9				13											
*25:05	A25								8	9			12	13							20				
*25:06	A25								8	9			12												
*26:01:01-26:01:20, 26:01:22, 26:08, 26:10-26:15, 26:17-26:18, 26:20, 26:23-26:29, 26:31-26:33, 26:35-26:43:02, 26:45-26:53, 26:55-26:63, 26:65-26:69	A26, A10, Null, –								8		10			13											
*26:01:21, 26:04, 26:34	A26, –								8		10														
*26:02	A26								8		10			w											
Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

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Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
HLA-A allele[4]	**ser**[5]																								
*26:03:01-26:03:02, 26:06, 26:21	A26, –		w						8			11		13										23	
*26:05	A26								8					13										23	
*26:07:01, 26:64	A26, –										10			13											
*26:07:02	A26		w								10			13											
*26:09	A26								8		10		12												
*26:16	A26							7			10			13											
*26:19	–				4									13	14										
*26:22	A26								8		10			13	14							21			
*26:30	A26		w						8					13										23	
*26:54	–								8		10		12	13							20				
*29:01:01:01-29:06, 29:08N-29:12, 29:15-29:18, 29:20-29:27, 29:29-29:31	A29, Null, –														14										
*29:07	A29						6								14										
*29:13	–														14			17							
*29:14	–														14		16								
*29:19	–														14					19					
*29:28	–								8						14										
*30:01:01-30:04:02, 30:06-30:07, 30:09-30:12, 30:14-30:15, 30:17-30:20, 30:22-30:43, 30:45, 30:47-30:54	A30, –, Null															15									
*30:13, 30:16, 30:44, 30:46	A30, –		w													15									
*30:08	A30				4											15									
*30:55	–													13		15									
*31:01:02-31:02, 31:05-31:07, 31:09-31:28, 31:30-31:34, 31:36-31:54	A31, Null, –																16								
*31:03	A31											11	12		14		16								
*31:04	A31												12		14		16								
*31:08	A31, A24					5											16								
*31:29	–						6										16								
*31:35	–															15	16	17							
*32:01:01-32:02, 32:06-32:12, 32:14, 32:16-32:36	A32, Null, –									9								17							
*32:03	A32																	17							
*32:04	A32, A3			3						9															
*32:05	A32					5											16	17							
*32:13	A32					5				9								17							
*32:15	A32								8	9								17							
Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

Negative Control

Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
HLA-A allele[4]	**ser**[5]																								
*33:01:01-33:01:06, 33:03:01-33:12, 33:14-33:18, 33:20-33:21, 33:23, 33:25-33:47, 33:50	A33, –																		18						Negative Control
*33:13, 33:48	–										10				14				18						
*33:19	–							7											18						
*33:22	–																		18			21			
*33:24	–																		18					23	
*33:49	–			w															18						
*33:51	–								8																
*34:01:01-34:01:02, 34:05-34:06	A34, A10								8			11	12												
*34:02:01, 34:03, 34:07	A34			3					8			11	12												
*34:02:02	–			w					8			11	12												
*34:04	A34			3					8			11	12		14										
*34:08	A34			3					8			11	12	13											
*34:09	–			3					8				12												
*36:01, 36:03, 36:05	A36, –	1																					22		
*36:02	A36	1		3																			22	23	
*36:04	A36	1			4																		22		
*43:01	A43												12	13											
*66:01, 66:04-66:08, 66:10-66:11, 66:13-66:15	A66, A26, –								8			11		13											
*66:02-66:03, 66:16	A66, A10, –								8				12												
*66:09	–								8			11		13	14							21			
*66:12	–								8					13											
*68:01:01:01-68:04, 68:06-68:12, 68:14, 68:16-68:19, 68:21:01-68:28, 68:31-68:44, 68:46-68:65, 68:67-68:70, 68:72-68:83	A68, A28, Null, –								8												20				
*68:05, 68:15, 68:20	A68		w						8												20			23	
*68:13, 68:66	A68				4				8												20				
*68:29	A68								8										18		20	21			
*68:30	A68		w						8												20				
*68:45	–							7	8												20				
*68:71	–								8					13							20				
*69:01	A69								8													21			
*74:01-74:06, 74:08-74:12N, 74:14N-74:15	A74, Null, –																			19					
*74:07	A74																	17		19					
*74:13	–													13						19					
Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

Well No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
HLA-A allele[4]	**ser[5]**																								
***80:01**	**A80**						**6**																	**23**	**N.C.**
***80:02**	**–**						**6**					**11**													
B*18:27							**6**																		
Well No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**

[1]The 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 low resolution SSP typing.
In addition, wells number 2, 4 to 6, 8, 9, 12 and 19 to 23 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.
[2]The nucleotide position, in the 1st, 2nd or 3rd exon, 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, in the 2nd or 3rd exon, 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.
[4]The sequence of the A*0105N has been shown to be identical to A*01:04N.
The A*01:34N allele has been renamed to A* 01:01:38L.
The A*020116 allele has been renamed to A*02:134.
The A*020120 allele has been renamed to A*02:01:18.
The sequence of the A*0223 allele has been shown to be identical to A*02:22:01.
The sequence of the A*0298 allele has been shown to be identical to A*02:96.
The A*02:100 allele has never been assigned.
The A*1128 allele has been renamed to A*11:15:02.
The sequence of the A*2401 allele has been shown to be in error.
The sequence of the A*2412 allele has been shown to be identical to A*24:08.
The A*2416 allele has been renamed to A*31:08.
The A*2465 allele has been renamed to A*24:13:02.
The A*26:44 allele has been renamed to A*26:43:02.
The sequence of the A*3005 allele has been shown to be identical to A*30:04.
The A*3021 allele has been renamed to A*30:11:02.
The sequence of the A*31011 allele has been shown to be identical to A*31:01:02.
The sequence of the A*3302 allele has been shown to be identical to A*33:03:01.
[5]The serological reactivity of all HLA-A alleles is not known. In this table we use the expert-assigned serological grouping in Tissue Antigens (2009) 73:95-170 and the serological grouping of the sequence-defined allele.
[6]The primer pairs in wells 1, 2, 11 and 15 will in many samples give rise to two or three HLA-specific PCR fragments.
[7]The A*23:14 and the A*24:05, 24:13:02 and 24:24 alleles will give rise to identical amplification patterns. These four alleles can be separated by the respective high resolution SSP primer sets.
[8]Primer mix 24 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', may be weakly amplified.

CELL LINE VALIDATION SHEET

HLA-A low resolution primer set

		IHWC cell line	A*	A*	Well 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		Lot No.:			201193101	201191802	201193103	201191804	201191805	201191806	201191807	201191808	201191809	201191810	201191811	201191812	201191813	201191814	201193115	201191816
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		-	+	-	-	-	-	W	-	-	-	-	-	-	-	-	-
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		-	+	-	-	-	-	W	-	-	-	-	-	-	-	-	-
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	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-

CELL LINE VALIDATION SHEET

HLA-A low resolution primer set

					Well						
					17	18	19	20	21	22	23
				Lot No.:	201191817	201191818	201194019	201191820	201191821	201191822	201191823
	IHWC cell line		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	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-A low resolution

Product number: **101.401-48/12 – including *Taq* polymerase**
Lot number: **94M**
Expiry date: **2014-May-01**
Number of tests: **48 tests – Product No. 101.401-48**
12 tests – Product No. 101.401-12
Number of wells per test: **23 + 1**

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2011-931-01	9	2011-918-09	17	2011-918-17
2	2011-918-02	10	2011-918-10	18	2011-918-18
3	2011-931-03	11	2011-918-11	19	2011-940-19
4	2011-918-04	12	2011-918-12	20	2011-918-20
5	2011-918-05	13	2011-918-13	21	2011-918-21
6	2011-918-06	14	2011-918-14	22	2011-918-22
7	2011-918-07	15	2011-931-15	23	2011-918-23
8	2011-918-08	16	2011-918-16		

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

Additional 5'- and 3'-primers in primer solutions 4, 5, 7, 8, 12, 14 to 17 and 20 to 23 were tested by separately adding 3'-primers, respectively 5'-primers.
One additional 5'-primer in primer solutions 1 and 10 was tested by separately adding one 3'-primer. Additional 3'-primers in primer solutions 3, 6, 18 and 19 were tested by separately adding one 5'-primer. One or two of the 5'-primers in primer solutions 2, 9, 10, 11 and 15 were not possible to test, and in primer solutions 3, 8, 18 and 19 one 3'-primer was not possible to test.

The negative control primer pairs, **Production No. 2011-928-01**, can detect contamination with PCR products diluted 10^{-7}.

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

Date of approval: 2011-December-09

Approved by:

Production Quality Control

Declaration of Conformity

Product name: *Olerup* SSP® HLA-A low resolution
Product number: 101.401-48/12
Lot number: 94M

Intended use: HLA-A low resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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Phone: +46-8-717 88 27
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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 9001:2008 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, Franzengatan 5, SE-112 51 Stockholm, Sweden.

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

Stockholm, Sweden
2011-December-09

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

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