Olerup SSP™ KIR Genotyping

Product number:	**104.101-12 – licensed for PCR** **104.101-12u – not licensed for PCR**
Lot number:	**X70**
Expiry date:	**2009-February-01**
Number of tests:	**12**
Number of tubes per test:	**23 + 1**
Storage - pre-aliquoted primers:	**dark at -20°C**
- PCR Master Mix:	**-20°C**

This Product Description is only valid for Lot No. X70.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP™ KIR GENOTYPING LOT

The KIR specificity and interpretation tables have been updated for the KIR alleles described since the previous *Olerup* SSP™ KIR Genotyping lot was made **(Lot No. N82)**.

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

Tube	5'-primer	3'-primer	rationale
3	-	Added	Primer added for the 2DL3*007 allele.

Well **24** contains Negative Control primer pairs, that will amplify more than 90% 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.

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'-Tg g3'**	**5'-Tg g3'**	**5'-Tg g3'**
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**
A*	**+**	**+**	**+**			
B*	**+**	**+**	**+**			
Cw*	**+**	**+**	**+**			
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 or the 2nd intron, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as in *Tissue Antigens* 1998, 51:II, 417-466. 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, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as in *Tissue Antigens* 1998, 51:II, 417-466. The sequence of the 3 terminal nucleotides of the primer is given.

PRODUCT DESCRIPTION

KIR Genotyping SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for KIR Genotyping.
The primer solutions are pre-aliquoted into 0.2 ml PCR tubes. Each tube in the set contains a dried primer solution consisting of a specific primer mix, i.e. allele- and group-specific primers as well as a ***control primer pair*** matching non-allelic sequences.
PCR Master Mix complete with Taq, *Taq* polymerase, nucleotides, buffer, glycerol and cresol red, as well as PCR lids are included in the licensed kit.
PCR Master Mix without Taq, nucleotides, buffer, glycerol and cresol red, as well as PCR lids are included in the unlicensed kit.
23 + 1 PCR reactions with a reaction volume of 10 µl are performed per sample.
Note: The pellets in the tubes may vary in form and colour. This does not affect the performance of the product.

PLATE LAYOUT

Each test consists of 24 PCR reactions in a 24 well cut PCR plate.

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 – KIR Genotyping primers.
Well 24 – Negative Control.
The 24 well cut PCR plate is marked with 'KIR X70'.
Well No. 1 is marked with '1'.
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.

UNIQUELY IDENTIFIED ALLELES

All the KIR alleles in the IPD KIR Sequence Database in November 2006[1] will be amplified by the primers in the KIR Genotyping SSP kit.

[1]KIR alleles listed on the IPD KIR web page 2006-November-10, release 1.3.0, www.ebi.ac.uk/ipd/kir.

LICENSES

104.101-12 – licensed for PCR.
Notice to purchaser: Limited License.

The purchase price of this product includes limited, non-transferable rights under U.S. Patents 4,683,202, 4,683,195 and 4,965,188 and their foreign counterparts, owned by Roche Molecular Systems, Inc. and F. Hoffman-La Roche Ltd ("Roche"), to use only this amount of the product to practice the Polymerase Chain Reaction ("PCR") Process described in said patents solely for the HLA Typing applications of the purchaser solely for organ or tissue or bone marrow transplantation, and explicitly excludes analysis of forensic evidence or parentage determination. The rights to use this product to perform and to offer commercial service for HLA Typing for organ or tissue transplantation using PCR, including reporting the results of the purchaser's activities for a fee or other commercial consideration, is also hereby granted. Further information on purchasing licenses to practice PCR may be obtained by contacting in the United States, the Director of Licensing at Roche Molecular Systems, inc., 1145 Atlantic Avenue, Alameda, California 94501, and outside the United States, the PCR Licensing Manager, F. Hoffmann-La Roche Ltd, Grenzacherstr. 124, CH-4070 Basel, Switzerland.

104.101-12u – not licensed for PCR.
Notice to purchaser: Disclaimer of License.

This product is optimized for use in the Polymerase Chain Reaction ("PCR") Process which is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd ("Roche"). No license under these patents to use the PCR Process is conveyed expressly or by implication to the purchaser of this product. Further information on purchasing licenses to practice PCR may be obtained by contacting in the United States, the Director of Licensing at Roche Molecular Systems, inc., 1145 Atlantic Avenue, Alameda, California 94501.

104.101-12 and 104.101-12u

These products use ARMS™ technology and is sold under license from Zeneca Limited. ARMS is the subject of European Patent No. 0332435, US Patent No. 5595890 and corresponding world-wide patents. ARMS is a trademark of Zeneca Limited.

GUARANTEE

Olerup SSP AB guarantees that the primers in the KIR Genotyping typing kit have the specificities given in the Specificity and Interpretation Tables of the product insert and in the GenoVision version of the HELMBERG-SCORE™ software.
When stored at –20°C, the dried primers are stable for 22 months from the date of manufacture.
When stored at –20°C, the PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* are stable for 24 months from the date of manufacture.
The kit is shipped at ambient temperature.

PROTOCOL

DNA EXTRACTION

Extracted, highly pure DNA is needed for SSP typings. We recommend isolation of DNA using GenoPrep B200 or GenoPrep B350 cartridges on the GenoM™-6 robotic workstation (GenoVision Europe *Tel:* +43 1 710 15 00 or GenoVision Inc. USA *Tel:* +1 610 430 88 41; http://www.genovision.com). Using GenoM™-6-extracted DNA ACD, EDTA and heparinised blood can be used as starting material. Because of its high purity, GenoM™-6-extracted DNA can be diluted when used in combination with *Olerup* SSP™ products. The recommended DNA concentration is 15 ng/ul.
Alternatively – BUT DO NOT USE HEPARINISED BLOOD WITH THESE METHODS - the DNA can be extracted using trimethylammoniumbromide salts (DTAB/CTAB) or by salting out. Dissolve the extracted DNA in dH_2O.

IMPORTANT:
Optimal DNA concentration using: GenoM™-6-extracted DNA, 15 ng/µl.
DNA extracted by other methods, 30 ng/µl.

Concentration exceeding 50 ng/µl will increase the risk for nonspecific amplifications and weak extra bands, especially for HLA Class I high resolution SSP typings.

PCR AMPLIFICATION

104.101-12 – licensed for PCR

For one KIR Genotyping typing, begin by adding to well No. 24, i.e. the well with the negative control primer pairs:

7 µl dH_2O
3 µl PCR Master Mix complete with *Taq*,

then add at room temperature in a 0.5 ml tube:

27 x 2 µl = 54 µl DNA (30 ng/µl)
27 x 3 µl = 81 µl PCR Master Mix complete with *Taq* – mix well before taking your aliquot
27 x 5 µl = 135 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-H_2O mixture into each of the 23 wells of an KIR Genotyping typing, i.e. wells 1 to 23. ***Well No. 1 of the 24 well PCR plate is marked with '1'.*** Close the 24 well PCR plate with the provided lids.

104.101-12u – not licensed for PCR

For one KIR Genotyping typing, begin by adding at room temperature in a 0.5 ml tube:

28 x 3 µl = 84 µl PCR Master Mix without *Taq* – mix well before taking your aliquot
2.2 µl *Taq* polymerase (5 units/µl)

Mix well, dispense 3 µl of the PCR Master Mix-*Taq* mixture from the 0.5 ml tube into well No. 24, i.e. the well with the negative control primer pairs. Then add 7 µl dH_2O to well 24.

Then add at room temperature to the 0.5 ml tube containing 84 + 2.2 - 3 = 83,2 µl PCR Master Mix-*Taq* mixture:

27 x 2 µl = 54 µl DNA (30 ng/µl)
27 x 5 µl – 2,2 µl = 132.8 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-*Taq*-H_2O mixture into each of the 23 wells of an KIR Genotyping typing, i.e. wells 1 to 23. ***Well No. 1 of the 24 well PCR plate is marked with '1'.*** Close the 24 well PCR plate with the provided lids.

Use a 96 well thermal cycler with a heated lid. The temperature gradient across the heating block should be < 1°C.

PCR cycling parameters:

1. 1 cycle	94°C	2 min	denaturation
2. 10 cycles	94°C	10 sec.	denaturation
	65°C	60 sec.	annealing and extension
3. 20 cycles	94°C	10 sec.	denaturation
	61°C	50 sec.	annealing
	72°C	30 sec.	extension

The same PCR cycling parameters are used for all the *Olerup* SSP kits.

AGAROSE GEL ELECTROPHORESIS

Prepare a 2% (w/v) agarose gel in 0.5 x TBE buffer. Dissolve the agarose by boiling in a microwave oven. Let the gel solution cool to 60°C. Stain the gel prior to casting with ethidium bromide (10 mg/ml), 5 µl per 100 ml gel solution. For maximal ease of handling use our ethidium bromide dropper bottles (Product No. 103.301-10), 1 drop of ethidium bromide solution per 50-75 ml of gel. **Note: Ethidium bromide is a powerful carcinogen.**
Load the PCR products, preferably using an 8-channel pipette. Load a DNA size marker (100 base pair ladder, Product No. 103.201-100) in one well per row.
Run the gel in 0.5 x TBE buffer, without re-circulation of the buffer, for 15-20 minutes at 8-10 V/cm.

DOCUMENTATION AND INTERPRETATION

Put the gel on a UV transilluminator and document by photography.
Record the presence and absence of specific PCR products. The relative lengths of the specific PCR products are helpful in the interpretation of the results.

Record the presence and relative lengths of the internal positive control bands. The differently sized control bands will help in the correct orientation of the typing as well as in kit identification.
Lanes without either control band or specific PCR products should be repeated.

Interpret the typings with the ***lot-specific Interpretation and Specificity Tables***.

INTERPRETATION SOFTWARE

The interpretation software (Product No. 110.101) can be helpful in the interpretation of the typings.

PCR MASTER MIXES

The PCR Master Mix complete with *Taq* contains:

Taq polymerase	0.4 unit per 10 µl SSP reaction
nucleotides	final concentration of each dNTP is 200 µM
PCR buffer	final concentrations: 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin
glycerol	final concentration of glycerol is 5%
cresol red	final concentration of cresol red is 100 µg/ml

The same PCR Master Mix complete with *Taq* is used for all the licensed *Olerup* SSP kits.

The PCR Master Mix without *Taq* contains:

nucleotides	final concentration of each dNTP is 200 µM
PCR buffer	final concentrations: 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin
glycerol	final concentration of glycerol is 5%
cresol red	final concentration of cresol red is 100 µg/ml

The same PCR Master Mix without *Taq* is used for all the unlicensed *Olerup* SSP kits.

The PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* can be shipped at ambient temperature.
When stored at –20°C, the PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* are stable for 24 months from the date of manufacture.
Vials with the PCR Master Mixes can be kept at +4°C for 4 weeks, but the PCR Master Mixes are then no longer stable for 24 months.

SPECIFICITY TABLE

KIR Genotyping SSP typing

Specificities and sizes of the PCR products of the 23 primer mixes used for KIR SSP Genotyping SSP.

Primer Mix	Approx. size of spec. PCR product[1]	Size of control band[2]	KIR Gene	Amplified KIR[3] alleles
1	145 bp	800 bp	2DL1	001-006
2	145 bp	800 bp	2DL2	001-005
3	520 bp	**1070 bp**	2DL3	001-007
4	200 bp	800 bp	2DL4	00101-011
5[5]	190 bp	800 bp	2DL5A, 2DL5B	001, 005 002-004, 006, 007
6[6]	1650 bp	**430 bp**	2DL5A	001, 005
7[6]	1650 bp	**515 bp**	2DL5B	002-004, 006, 007
8[4]	100 bp	800 bp	2DS1	001-004
9	205 bp	800 bp	2DS2	00101-005
10	160 bp	800 bp	2DS3	00101-002
11	215 bp	800 bp	2DS4	00101-00102
12	200 bp	800 bp	2DS4	003, 004, 006, 007
13[4]	110 bp	800 bp	2DS5	001-005
14	130 bp	800 bp	3DL1	00101-002, 00401-009, 01501-020
15[4]	95 bp	800 bp	3DL2	001-016
16[4]	115 bp	800 bp	3DL3	001-022
17	140 bp	800 bp	3DS1 3DL1	010-014 009
18	165 bp	800 bp	2DP1	00101-002
19[4]	125 bp	800 bp	3DP1	001-004
20	235 bp	800 bp	3DP1	00301-00301, 004?
21	145 bp	800 bp	2DS1	001
22	145 bp	800 bp	2DS1	002-004
23[4]	80 bp	800 bp	3DL1	00401-00402

[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 KIR Genotyping 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 band may sometimes be observed. Such bands can be disregarded and do not influence the interpretation of the SSP typings.
[2]The internal positive control primer pairs amplify segments of the human growth hormone gene. The three different control primer pairs give rise to either an internal positive control band of 800

base pairs, for most tubes, or a band of 1070 base pairs or 430 base pairs, for some tubes.
Tube number 3 contains the primer pair giving rise to the 1070 bp internal positive control bane, tube number 6 contains the primer pair giving rise to the 430 base pair internal positive control band and tube number 7 contains the primer pair giving rise to the 515 base pair internal positive control band in order to help in the correct orientation and kit identification of the KIR Genotyping typing.

PLEASE NOTE: All the SSP kits, except the B*37, B*41, B*42, B*46, B*47, B*48, B*49, B*50, B*53, B*67, B*78, B*81 and B*82 kits and the Cw*01, Cw*02, Cw*08, Cw*12,Cw*14, Cw*15, Cw*16, Cw*17 and Cw*18 kits, from *Olerup* SSP AB can be uniquely identified by the number of tubes and the kit-specific pattern of the two differently sized control bands.

In the presence of a specific amplification the intensity of the control band often decreases.
[3]KIR alleles listed on the IPD KIR web page 2006-November-10, release 1.3.0, www.ebi.ac.uk/ipd/kir.
[4]Short specific PCR fragments are less intense and not as sharp as longer specific bands.
[5]Primer mix 5 may yield somewhat less specific PCR product than the other KIR SSP primer mixes.
[6]The specific PCR product generated by primer mixes 6 and 7 are longer than the internal positive control band.
'?', the 2nd intron sequence of the primer matching region is not know.

INTERPRETATION TABLE

KIR SSP Genotyping

Amplification patterns of the KIR alleles

	Tube											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	145	145	520	200	190	1650	1650	100	205	160	215	200
PCR product												
Length of int.	800	800	1070	800	800	430	515	800	800	800	800	800
pos. control[1]												
5'-primer(s)[2]	130	208	344	208	226	-16	-16	165	140	226	229	234
	5'-gAA3'	5'-CCA3'	5'-CTg3'	5'-CCg3'	5'-CCA3'	5'-TCA3'	5'-TCg3'	5'-gAg3'	5'-gTA3'	5'-CCT3'	5'-CTA3'	5'-TCT3'
								165				
								5'-gAA3'				
3'-primer(s)[3]	165	243	350	262	276	27	27	185	195	266	288	288
	5'-gCg3'	5'-ACA3'	5'-CAA3'	5'-ggA3'	5'-gAg3'	5'-ACA3'	5'-gTT3'	5'-gAC3'	5'-ATg3'	5'-CCT3'	5'-ggA3'	5'-ggA3'
			351									
			5'-ACC3'									
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
KIR allele[4]												
2DL1*001-006	+											
2DL2*001-005		+										
2DL3*001-007			+									
2DL4*00101-011				+								
2DL5A*001, 005					+	+						
2DL5B*002-004, 006, 007					+		+					
2DS1*001								+				
2DS1*002-004								+				
2DS2*00101-005									+			
2DS3*00101-002										+		
2DS4*0010101-00102											+	
2DS4*003, 004, 006, 007												+
2DS5*001-005												
3DL1*00101-002, 00501-008, 01501-020												
3DL1*009												
3DL1*00401-00402												
3DL2*001-016												
3DL3*001-022												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE

KIR SSP Genotyping

Amplification patterns of the KIR alleles

Tube											
13	14	15	16	17	18	19	20	21	22	23	
110	130	95	115	140	165	125	235	145	145	80	Length of spec. PCR product
800	800	800	800	800	800	800	800	800	800	800	Length of int. pos. control[1]
142	136	27	156	58	29	25	2nd I	130	130	31	5'-primer(s)[2]
5'-ACC3'	5'-CAA3'	5'-TCA3'	5'-CCC3'	5'-Agg3'	5'-CAT3'	5'-Tgg3'	5'-gCC3'	5'-gAA3'	5'-gAA3'	5'-TCA3'	
165	166	45	181	92	71	54	54	165	165	44	3'-primer(s)[3]
5'-gTg3'	5'-CAA3'	5'-ggC3'	5'-gTA3'	5'-CAT3'	5'-TAC3'	5'-TAC3'	5'-TAC3'	5'-gCC3'	5'-gCT3'	5'-TCC3'	
13	14	15	16	17	18	19	20	21	22	23	Tube No.
											KIR allele[4]
											2DL1*001-006
											2DL2*001-005
											2DL3*001-007
											2DL4*00101-011
											2DL5A*001, 005
											2DL5B*002-004, 006, 007
								+			2DS1*001
									+		2DS1*002-004
											2DS2*00101-005
											2DS3*00101-002
											2DS4*0010101-00102
											2DS4*003, 004, 006, 007
+											2DS5*001-005
	+										3DL1*00101-002, 00501 008, 01501-020
	+			+							3DL1*009
	+									+	3DL1*00401-00402
		+									3DL2*001-016
			+								3DL3*001-022
13	14	15	16	17	18	19	20	21	22	23	Tube No.

Length of spec.	145	145	520	200	190	1650	1650	100	205	160	215	200
PCR product												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
3DS1*010-014												
2DP1*00101-002												
3DP1*001-002												
3DP1*00301-00302												
3DP1*004												
KIR allele[4]												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12

[1]The internal positive control primer pairs amplify segments of the human growth hormone gene. The three different control primer pairs give rise to either an internal positive control band of 800 base pairs, for most tubes, or a band of 1070 base pairs or 430 base pairs, for some tubes.
Tube number 3 contains the primer pair giving rise to the 1070 bp internal positive control band and tubes number 6 and 7 contain the primer pair giving rise to the 430 base pair internal positive control band in order to help in the correct orientation and kit identification of the KIR Genotyping typing.

PLEASE NOTE: All the SSP kits, except the B*37, B*41, B*42, B*46, B*47, B*48, B*49, B*50, B*53, B*67, B*78, B*81 and B*82 kits and the Cw*01, Cw*02, Cw*08, Cw*12,Cw*14, Cw*15, Cw*16, Cw*17 and Cw*18 kits, from *Olerup* SSP AB can be uniquely identified by the number of tubes and the kit-specific pattern of the two differently sized control bands.

110	130	95	115	140	165	125	235	145	145	80	Length of spec.
											PCR product
13	14	15	16	17	18	19	20	21	22	23	Tube No.
				+							3DS1*010-014
					+						2DP1*00101-002
						+					3DP1*001-002
						+	+				3DP1*00301-00302
						+	?				3DP1*004
											KIR allele[4]
13	14	15	16	17	18	19	20	21	22	23	Tube No.

[2]The nucleotide position, in the 1st, 3rd, 4th, 5th or 7th exon or the 2nd intron matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the KIR web page 2006-November-10, release 1.3.0, www.ebi.ac.uk/ipd/kir. The sequence of the 3 terminal nucleotides of the primer is given.
[3]The nucleotide position, in the 3rd, 4th, 5th or 8th exon, matching the specificity-determining 3'-end of the primer. Nucleotide numbering as on the KIR web page 2006-November-10, release 1.3.0, www.ebi.ac.uk/ipd/kir. The sequence of the 3 terminal nucleotides of the primer is given in the anti-sense direction.
[4]KIR alleles listed on the IPD KIR web page 2006-November-10, release 1.3.0, www.ebi.ac.uk/ipd/kir.
'?', the 2nd intron sequence of the primer matching region is not know.

CELL LINE VALIDATION SHEET

KIR Genotyping primer set

			Tube														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
			200505001	200505002	200733003	200505004	200505005	200505006	200505007	200505008	200505009	200505010	200505011	200505012	200505013	200505014	200505015	200505016
	cell line																	
1	9001	SA	+	-	+	+	-	-	-	-	-	-	+	-	-	+	+	+
2	9280	LK707	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+
3	9011	E4181324	+	-	+	+	-	-	-	-	-	-	+	+	-	+	+	+
4	9275	GU373	+	-	+	+	-	-	-	-	-	-	+	+	-	+	+	+
5	9009	KAS011	+	-	+	+	+	+	-	+	-	-	-	+	+	+	+	+
6	9353	SM	+	-	+	+	+	+	-	+	-	+	+	-	-	+	+	+
7	9020	QBL	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
8	9007	DEM	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
9	9026	YAR	+	-	+	+	-	-	-	-	-	-	+	+	-	+	+	+
10	9107	LKT3	+	-	+	+	-	-	-	-	-	-	+	-	-	+	+	+
11	9051	PITOUT	+	+	+	+	-	-	-	-	+	-	-	+	-	+	+	+
12	9052	DBB	-	+	+	+	+	-	+	-	+	+	-	+	-	+	+	+
13	9067	BTB	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+
14	9071	OLGA	+	-	+	+	+	+	-	+	-	-	-	+	+	+	+	+
15	9075	DKB	+	-	+	+	-	-	-	-	-	-	+	-	-	+	+	+
16	9037	SWEIG007	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+
17	9008	WILJON	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+
18	9257	32367	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+
19	9038	BM16	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+
20	9059	SLE005	+	-	+	+	-	-	-	-	-	-	+	+	-	+	+	+
21	9064	AMALA	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	+
22	9056	KOSE	+	+	+	+	-	-	-	-	+	-	-	+	-	+	+	+
23	9124	IHL	+	+	+	+	-	-	-	-	+	-	+	+	-	+	+	+
24	9035	JBUSH	+	-	+	+	-	-	-	-	-	-	+	+	-	+	+	+
25	9049	IBW9	+	-	+	+	-	-	-	-	-	+	+	+	-	+	+	+
26	9285	WT49	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+
27	9191	CH1007	+	+	+	+	+	-	+	-	+	-	+	+	-	+	+	+
28	9320	BEL5GB	+	+	-	+	+	-	+	-	+	-	-	+	-	+	+	+
29	9050	MOU	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+
30	9021	RSH	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+
31	9019	DUCAF	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+
32	9297	HAG	+	-	+	+	-	-	-	-	-	-	+	+	-	+	+	+
33	9098	MT14B	+	-	+	+	-	-	-	-	-	-	+	-	-	+	+	+
34	9104	DHIF	+		+	+	-	-	-	-	+	-	+	+	-	+	+	+
35	9302	SSTO	+	+	+	+	-	-	-	-	+	-	+	+	-	+	+	+
36	9024	KT17	+	-	+	+	+	+	-	+	-	+	-	+	-	+	+	+
37	9065	HHKB	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+
38	9099	LZL	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+
39	9315	CML		+	-	+	+	+	+	+	+	+	-	+	-	+	+	+
40	9134	WHONP199	+	-	+	+	-	-	-	-	-	-	+	-	-	+	+	+
41	9055	H0301	+	+	-	+	+	-	+	-	+	+	+	-	-	+	+	+
42	9066	TAB089	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+
43	9076	T7526	+	-	+	+	+	+	-	+	+	-	+	-	+	+	+	+
44	9057	TEM	+	+	+	+	+	-	+	-	+	+	-	+	-	+	+	+
45	9239	SHJO	+	+	+	+	+	-	+	-	-	-	+	-	+	+	+	+
46	9013	SCHU	+	-	+	+	-	-	-	-	-	-	+	+	-	+	+	+
47	9045	TUBO	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+
48	9303	TER-ND	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+

CELL LINE VAL. SHEET

KIR Genotyping primer set

			Tube						
			17	18	19	20	21	22	23
			200505017	200505018	200505019	200505020	200505021	200505022	200505023
	cell line								
1	9001	SA	-	+	+	+	-	-	-
2	9280	LK707	-	-	+	-	-	+	-
3	9011	E4181324	+	+	+	+	-	-	+
4	9275	GU373	-	+	+	+	-	-	-
5	9009	KAS011	+	+	+	+	-	+	-
6	9353	SM	+	+	+	+	-	+	-
7	9020	QBL	+	+	+	+	-	+	+
8	9007	DEM	+	+	+	+	-	+	-
9	9026	YAR	-	+	+	+	-	-	-
10	9107	LKT3	-	+	+	+	-	-	-
11	9051	PITOUT	-	+	+	+	-	-	+
12	9052	DBB	-	+	+	+	-	-	+
13	9067	BTB	-	+	+	+	-	-	+
14	9071	OLGA	+	+	+	+	-	+	-
15	9075	DKB	-	+	+	+	-	-	-
16	9037	SWEIG007	-	+	+	+	-	-	+
17	9008	WILJON	-	+	+	+	-	-	+
18	9257	32367	-	+	+	+	-	-	+
19	9038	BM16	-	+	+	+	-	-	-
20	9059	SLE005	-	+	+	+	-	-	-
21	9064	AMALA	+	+	+	+	-	+	-
22	9056	KOSE	-	+	+	+	-	-	+
23	9124	IHL	-	+	+	+	-	-	+
24	9035	JBUSH	-	+	+	+	-	-	-
25	9049	IBW9	-	+	+	+	-	-	+
26	9285	WT49	-	+	+	+	-	+	-
27	9191	CH1007	-	+	+	+	-	-	-
28	9320	BEL5GB	-	+	+	+	-	-	+
29	9050	MOU	-	+	+	+	-	-	+
30	9021	RSH	-	+	+	+	-	-	-
31	9019	DUCAF	-	+	+	+	-	-	-
32	9297	HAG	-	+	+	+	-	-	-
33	9098	MT14B	-	+	+	+	-	-	-
34	9104	DHIF	-	+	+	+	-	-	-
35	9302	SSTO	-	+	+	+	-	-	-
36	9024	KT17	+	+	+	+	-	+	-
37	9065	HHKB	+	+	+	+	-	+	-
38	9099	LZL	-	+	+	+	-	+	-
39	9315	CML	+	+	+	+	-	+	+
40	9134	WHONP199	-	+	+	+	-	-	-
41	9055	H0301	-	+	+	+	-	-	-
42	9066	TAB089	-	+	+	+	-	-	-
43	9076	T7526	+	+	+	+	-	+	-
44	9057	TEM	-	+	+	+	-	-	-
45	9239	SHJO	-	+	+	+	-	-	-
46	9013	SCHU	-	+	+	+	-	-	-
47	9045	TUBO	-	+	+	+	-	-	-
48	9303	TER-ND	-	+	+	+	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP™ KIR Genotyping SSP

Product number: **104.101-12 – licensed for PCR**
104.101-12u – not licensed for PCR
Lot number: **X70**
Expiry date: **2009-February-01**
Number of tests: **12**
Number of tubes per test: **23 + 1**

Tube specifications:

Tube No.	Production No.	Tube No.	Production No.	Tube No.	Production No.
1	2005-050-01	9	2005-050-09	17	2005-050-18
2	2005-050-02	10	2005-050-10	18	2005-050-19
3	2007-330-03	11	2005-050-12	19	2005-050-20
4	2005-050-04	12	2005-050-13	20	2005-050-21
5	2005-050-05	13	2005-050-14	21	2005-050-22
6	2005-050-06	14	2005-050-15	22	2005-050-23
7	2005-050-07	15	2005-050-16	23	2005-050-24
8	2005-050-08	16	2005-050-17		

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

The negative control primer pairs, **Production No. 2006-257-01**, can detect contamination with PCR products diluted 10^{-7}.

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

Date of approval: 2007-April-18

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP™ KIR Genotyping
Product number: 104.101-12, 104.101-12u
Lot number: X70

Intended use: KIR Genotyping

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 III, 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.

Saltsjöbaden, Sweden
2007-April-18

Olle Olerup
Managing Director

WARRANTY

Olerup SSP AB warrants its products to the original purchaser against defects in materials and workmanship under normal use and application. *Olerup* SSP AB's sole obligation under this warranty shall be to replace, at no charge, any product that does not meet the performance standards stated on the product specification sheet.

This warranty applies only to products that have been handled and stored in accordance with *Olerup* SSP AB's recommendations, and does not apply to products that have been the subject of alternation, misuse, or abuse.

All claims under this warranty must be directed to *Olerup* SSP AB in writing and must be accompanied by a copy of the purchaser's invoice. This warranty is in lieu of all other warranties, expressed or implied, including the warranties of merchantability and fitness for a particular purpose. In no case shall *Olerup* SSP AB be liable for incidental or consequential damages.

This product may not be reformulated, repacked or resold in any form without the written consent of *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

Handle all samples as if capable of transmitting disease. All work should be performed wearing gloves and appropriate protection.

Olerup SSP™ is a trademark of *Olerup* SSP AB.
PCR™ is a trademark of F. Hoffmann-La Roche Ltd.
ARMS™ is a trademark of Zeneca Ltd.

ADDRESSES:

Manufacturer:
***Olerup* SSP AB**, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.
Tel: +46-8-717 88 27
Fax: +46-8-717 88 18
E-mail: info-ssp@olerup.com
Web page: http://www.olerup.com

Distributed by:
Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.
Tel: +43-1-710 15 00
Fax: +43-1-710 15 00 10
E-mail: support-at@olerup.com
Web page: http://www.olerup.com

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382
Tel: 1-877-OLERUP1
Fax: 610-344-7989
E-mail: info.us@olerup.com
Web page: http://www.olerup.com

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.