Olerup SSP® KIR Genotyping

Product number:	**104.101-12 – including *Taq* polymerase** **104.101-12u – without *Taq* polymerase**
Lot number:	**35F**
Expiry date:	**2011-January-01**
Number of tests:	**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. 35F.

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

The KIR Genotyping primer set and specificity and interpretation tables are unchanged compared to the previous *Olerup* SSP® KIR Genotyping lot **(Lot No. 71E)**.

Changes in revision R02 compared to R01:
1. The sizes of the specific PCR products in wells 5 and 22 have been corrected to 155 and 95 bp, respectively.

Changes in revision R03 compared to R02:
1. In primer mix 2, the positive control band may be weaker than for other KIR primer mixes.

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'-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, 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

KIR Genotyping SSP typing

CONTENT
The primer set contains 5'- and 3'-primers for KIR Genotyping.

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 GENOTYP'.
Well No. 1 is marked with the Lot No. '35F'.
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 available in the IPD KIR Sequence Database in January 2008[1] will be amplified by the primers in the KIR Genotyping SSP kit.

[1]KIR alleles listed on the IPD KIR web page 2008-January-18, release 2.0.0, www.ebi.ac.uk/ipd/kir.

PROTOCOL

DNA EXTRACTION

Extracted, highly pure DNA is needed for SSP typings. DNA samples to be used for PCR-SSP HLA typing should be re-suspended in dH_2O. The A260/A280 ratio should be 1.6 – 2.0 by UV spectrophotometry for optimal band visualization during electrophoresis.

We recommend automated DNA extraction with the QIAGEN EZ1 DSP DNA Blood System. ACD blood should be used as starting material.

Alternatively, the DNA can be extracted by any preferred method yielding pure DNA. When using alternative methods, the DNA concentration should be adjusted to 30 ng/µl. ***Do not use heparinised blood with these methods.***

Recommended DNA concentration using:
EZ1-extracted DNA, 15 ng/µl.
DNA extracted by other methods, 30 ng/µl.

Concentrations exceeding 50 ng/µl will increase the risk for nonspecific amplifications and weak extra bands, especially for HLA Class I high resolution SSP typings. If necessary, dilute the extracted DNA in dH_2O.

DNA samples should not be re-suspended in solutions containing chelating agents such as EDTA, above 0.5 mM in concentration.

DNA samples may be used immediately after extraction or stored at +4°C for up to 2 weeks with no adverse effects on results. DNA samples can be stored at -20°C or colder for 9 months. The purity and concentration of extracted DNA samples that have been stored for a prolonged period should be tested for acceptability prior to HLA typing.

DNA samples should be shipped at +4°C or colder to preserve their integrity during transport.

PCR AMPLIFICATION

104.101-12 – including Taq *polymerase*

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. Cover the primer tray(s) with the provided adhesive seals. Check that all reaction wells are completely covered to prevent evaporative loss during PCR amplification.

104.101-12u – without *Taq* polymerase

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. Cover the primer tray(s) with the provided adhesive seals. Check that all reaction wells are completely covered to prevent evaporative loss during PCR amplification.

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* polymerase 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 *Olerup* SSP kits.

The PCR Master Mix without *Taq* polymerase 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 *Olerup* SSP kits.

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	Size of spec. PCR product[1]	Size of control band[2]	KIR Gene	Amplified KIR[3] alleles
1	145 bp	1070 bp	2DL1	001-010
2[7]	145 bp	1070 bp	2DL2	001-005
3	520 bp	1070 bp	2DL3	001-007
4	200 bp	1070 bp	2DL4	00101-012
5[5]	155 bp	1070 bp	2DL5A, 2DL5B	0010101-0010102, 0050101-005010102 0020101-004, 00601-009
6[6]	1650 bp	**430 bp**	2DL5A	0010101-0010102, 0050101-005010102
7[6]	1650 bp	**515 bp**	2DL5B	0020101-004, 00601-000
8[4]	100 bp	1070 bp	2DS1	001-004
9	205 bp	1070 bp	2DS2	0010101-005
10	160 bp	1070 bp	2DS3	00101-004
11	215 bp	1070 bp	2DS4	0010101-00103
12	200 bp	1070 bp	2DS4	003, 004, 006, 007, 009
13[4]	110 bp	1070 bp	2DS5	001-008
14	130 bp	1070 bp	3DL1	00101-002, 00401-009, 01501-044, 056, 057
15[4]	95 bp	1070 bp	3DL2	00101-021
16[4]	115 bp	1070 bp	3DL3	00101-031
17	130 bp	1070 bp	3DS1	010-014, 045-049N, 055
18	165 bp	1070 bp	2DP1	00101-003
19[4]	125 bp	1070 bp	3DP1	001-006
20	235 bp	1070 bp	3DP1	00301-00301, 004-006[?]
21	145 bp	1070 bp	2DS1	001
22[4]	95 bp	1070 bp	2DS1	0020101-004
23[4]	80 bp	1070 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 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 length 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 control primer pair gives rise to an internal positive control band of 1070 base pairs for most wells. Well number 6 contains the primer pair giving rise to the 430 base pair internal positive control band and well 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.
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 2008-January-18, release 2.0.0, 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.
[7]In primer mix 2, the positive control band may be weaker than for other KIR primer mixes.
'?', the 2nd intron sequence of the primer matching region is not know.

INTERPRETATION TABLE

KIR SSP Genotyping

Amplification patterns of the KIR alleles

	Well											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	145	145	520	200	155	1650	1650	100	205	160	215	200
PCR product												
Length of int.	1070	1070	1070	1070	1070	430	515	1070	1070	1070	1070	1070
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'									
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
KIR allele[4]												
2DL1*001-010	1											
2DL2*001-005		2										
2DL3*001-007			3									
2DL4*00101-012				4								
2DL5A*0010101-0010102, 0050101-05010102					5	6						
2DL5B*0020101-004, 00601-009					5		7					
2DS1*001								8				
2DS1*0020101-004								8				
2DS2*0010101-005									9			
2DS3*00101-004										10		
2DS4*0010101-00103											11	
2DS4*003, 004, 006, 007, 009												12
2DS5*001-008												
3DL1*00101-002, 00501-009, 01501-044, 056, 057												
3DL1*00401-00402												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE

KIR SSP Genotyping

Amplification patterns of the KIR alleles

Well											
13	14	15	16	17	18	19	20	21	22	23	
110	130	95	115	130	165	125	235	145	95	80	Length of spec. PCR product
1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	Length of int. pos. control[1]
142	136	27	156	133	29	25	2nd I	130	130	31	5'-primer(s)[2]
5'-ACC3'	5'-CAA3'	5'-TCA3'	5'-CCC3'	5'-TCT3'	5'-CAT3'	5'-Tgg3'	5'-gCC3'	5'-gAA3'	5'-gAA3'	5'-TCA3'	
165	166	45	181	163	71	54	54	165	165	44	3'-primer(s)[3]
5'-gTg3'	5'-CAA3'	5'-ggC3'	5'-gTA3'	5'-ggA3'	5'-TAC3'	5'-TAC3'	5'-TAC3'	5'-gCC3'	5'-gCT3'	5'-TCC3'	
13	14	15	16	17	18	19	20	21	22	23	Well No.
											KIR allele[4]
											2DL1*001-010
											2DL2*001-005
											2DL3*001-007
											2DL4*00101-012
											2DL5A*0010101-0010102, 0050101-05010102
											2DL5B*0020101-004, 00601-009
								21			2DS1*001
									22		2DS1*0020101-004
											2DS2*0010101-005
											2DS3*00101-004
											2DS4*0010101-00103
											2DS4*003, 004, 006, 007, 009
13											2DS5*001-008
	14										3DL1*00101-002, 00501-009, 01501-044, 056, 057
	14									23	3DL1*00401-00402
13	14	15	16	17	18	19	20	21	22	23	Well No.

Length of spec.	**145**	**145**	**520**	**200**	**155**	**1650**	**1650**	**100**	**205**	**160**	**215**	**200**
PCR product												
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
3DL2*00101-021												
3DL3*00101-031												
3DS1*010-014, 045-049N, 055												
2DP1*00101-003												
3DP1*001-002												
3DP1*00301-00302												
3DP1*004, 005, 006												
KIR allele[4]												
Well 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 control primer pair gives rise to an internal positive control band of 1070 base pairs for most wells. Well number 6 contains the primer pair giving rise to the 430 base pair internal positive control band and well 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.

110	130	95	115	130	165	125	235	145	95	80	Length of spec.
											PCR product
13	14	15	16	17	18	19	20	21	22	23	Well No.
		15									3DL2*00101-021
			16								3DL3*00101-031
				17							3DS1*010-014, 045-049N, 055
					18						2DP1*00101-003
						19					3DP1*001-002
						19	20				3DP1*00301-00302
						19	?				3DP1*004, 005, 006
											KIR allele[4]
13	14	15	16	17	18	19	20	21	22	23	Well 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 2008-January-18, release 2.0.0, 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

			Well															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
			200853801	200853802	200853803	200853804	200853805	200853806	200853807	200853808	200853809	200853810	200853811	200853812	200853813	200853814	200853815	200853816
	IHWC 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

			Well						
			17	18	19	20	21	22	23
			200853817	200853818	200853819	200853820	200853821	200853822	200853823
	IHWC 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 – including *Taq* polymerase**
104.101-12u – without *Taq* polymerase
Lot number: **35F**
Expiry date: **2011-January-01**
Number of tests: **12**
Number of wells per test: **23 + 1**

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2008-538-01	9	2008-538-09	17	2008-538-18
2	2008-538-02	10	2008-538-10	18	2008-538-19
3	2008-538-03	11	2008-538-12	19	2008-538-20
4	2008-538-04	12	2008-538-13	20	2008-538-21
5	2008-538-05	13	2008-538-14	21	2008-538-22
6	2008-538-06	14	2008-538-15	22	2008-538-23
7	2008-538-07	15	2008-538-16	23	2008-538-24
8	2008-538-08	16	2008-538-17		

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

The negative control primer pairs, **Production No. 2008-417-01**, can detect contamination with PCR products diluted 10^{-7}.

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

Date of approval: 2010-March-24

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® KIR Genotyping
Product number: 104.101-12/12u
Lot number: 35F

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 9001:2008 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
2010-March-12

Olle Olerup
Managing Director

TRADEMARKS USED IN THIS DOCUMENT/PRODUCT

Olerup SSP® is a registered trademark of *Olerup* SSP AB.
Qiagen™ is a trademark of QIAGEN.
Helmberg-SCORE™ is a trademark of W.M.C. Helmberg
ARMS™ is a trademark of AstraZeneca UK Ltd.

PATENTS USED IN THIS DOCUMENT/PRODUCT

These products use ARMS™ technology and is sold under license from AstraZeneca UK Ltd. ARMS is the subject of European Patent No. 0 332 435 B1, US Patent No. 5 595 890, Canadian Patent No. 1 323 591 and corresponding worldwide patents. ARMS is a trademark of AstraZeneca UK Ltd.

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.

GUARANTEE

Olerup SSP AB guarantees that the primers in the *Olerup* SSP® typing trays have the specificities given in the lot-specific Specificity and Interpretation Tables of the product insert and in the Helmberg-SCORE™ software.
When stored at –20°C, the dried primers are stable for 24 months from the date of manufacture.
When stored at –20°C, the PCR Master Mix including *Taq* polymerase and the PCR Master Mix without *Taq* polymerase are stable for 27 months from the date of manufacture.

EUROPEAN AUTHORIZED REPRESENTATIVE

The Authorized Representative located within the European Community is: *Olerup* SSP AB, Hasselstigen 1, SE-133 33, Sweden.

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-ssp.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**.