Olerup SSP® KIR Genotyping

Product number:	**104.101-12 – including *Taq* polymerase** **104.101-12u – without *Taq* polymerase**
Lot number:	**7N4**
Expiry date:	**2026-11-01**
Number of tests:	**12**
Number of wells per test:	**29+1**
Storage - pre-aliquoted primers:	**dark, between -15°C and -25°C**
- PCR Master Mix:	**between -15°C and -25°C**
- Adhesive PCR seals	**RT**

This Product Description is only valid for Lot No. 7N4.

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

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® KIR GENOTYPING LOT (4N4)

- The KIR Genotyping kit design, specificity and interpretation tables are based on IPD-KIR database 2.11.0

The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot **(Lot No. 4N4)**.

Well	5'-primer	3'-primer	rationale
6	Added	Added	Optimized 2DL5-specific primers.
14	Exchanged	Exchanged, removed	Exchanged primer pair for improved allelic resolution. Removed redundant 3'-primer.

Well **30** contains Negative Control primer pairs, that will produce exon 4 and/or exon 5 amplicons for more than 97% of applicable KIR alleles as well as amplicons generated by positive control primer pairs constituent of all primer mixes in the Olerup SSP® product range.

PCR product sizes: 280bp KIR specific amplicons
430bp Positive control

Length of PCR product	**280**	**280**	**280**	**280**
5'-primer[1]	**110**	**109**	**208**	**208**
	5'-CAg3'	**5'-CCT3'**	**5'-CCA3'**	**5'-CCg3'**
3'-primer	**187**	**187**	**288**	**288**
	5'-ggT3'	**5'-ggT3'**	**5'-gTC3'**	**5'-gTC3'**
	187	**187**	**288**	**288**
	5'-ggT3'	**5'-ggT3'**	**5'-ggT3'**	**5'-ggT3'**
			288	**288**
			5'-gAT3'	**5'-gAT3'**
2DL1*	**+**		**+**	
2DL2*	**+**		**+**	
2DL3*	**+**		**+**	
2DL4*	**N/A**	**N/A**		**+**
2DL5A*	**N/A**	**N/A**	**+**	
2DL5B*	**N/A**	**N/A**	**+**	
2DS1*	**+**		**+**	
2DS2*	**+**		**+**	
2DS3*	**+**		**+**	
2DS4*		**+**	**+**	
2DS5*	**+**		**+**	
3DL1*	**+**		**+**	
3DL2*	**+**		**+**	
3DL3*	**+**		**+**	
3DS1*	**+**		**+**	
2DP1*	**+**		**+**	
3DP1*	**+**		**+**	

[1]The codon position for KIR genes, in the 4th or 5th exon, matching the specificity-determining 3'-end of the primer is given. Codon numbering as on the www.ebi.ac.uk/ipd/kir web site. The sequence of the 3 terminal nucleotides of the primer is given.

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PRODUCT DESCRIPTION

KIR Genotyping SSP typing

CONTENT

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

PLATE LAYOUT

Each test consists of 30 PCR reactions in a 32 well cut PCR plate. Wells 31 and 32 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	NC	empty	empty

The 32 well cut PCR plate is marked with 'KIR GENOTYP' in silver/gray ink.
Well No. 1 is marked with the Lot No. '7N4'.
Wells 1 to 29 – KIR Genotyping primers.
Well 30 – Negative Control.
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 32 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 December 2021[1,2] will be amplified by the primers in the KIR Genotyping SSP kit.

[1]KIR alleles listed on the IPD KIR web page 2021-December-16, release 2.11.0, www.ebi.ac.uk/ipd/kir.
[2]Primer mix 8 does not amplify the 2DS1*013 allele. Due to sequence homology between allele groups this allele is amplified in primer mixes 1 and 29. Hence, a sample that is positive for 2DL1 and 2DP1*006, 009 or 010 may be falsely interpreted as 2DS1-positive. 2DS1*013 is a rare, unconfirmed allele. Caution should be used when interpreting these results.

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. 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

INSTRUMENT REQUIREMENTS

A thermocycler with the following minimum specifications should be used:

- heated lid with a temperature of 104°C for oil-free operation
- sample block (aluminum, silver, or gold-plated silver) for use with either a 96-well PCR plate or 0.2 ml thin-walled reaction tubes
- Olerup SSP kits are validated on the following cyclers.

Recommended ramp rates:

- GeneAmp 9700: GeneAmp 9700 cycler set to the 9600 mode. This corresponds to a **sample ramp rate** of 1.6°C/s up and 0.8°C/s down.
- ProFlex 1x96-well block: ProFlex PCR cycler with a block ramp rate of 3.0°C/s (each step 3.0°C/s). A **block ramp rate** of 3.0°C/s correspond to a sample ramp rate of 1.52°C/s up and 1.36°C/s down.
- ProFlex 2x96-well block: ProFlex PCR cycler with a block ramp rate of 3.0°C/s (each step 3.0°C/s). A **block ramp rate** of 3.0°C/s correspond to a sample ramp rate of 1.9°C/s up and 1.6°C/s down.

Note: Higher ramp rates than the equivalent to the described may have an effect on the typing results. Please also note that the effect on the typing may differ between different non-validated cyclers depending on the settings.

- temperature range of 4.0°C to 99.9°C
- temperature accuracy of ±0.25°C over the range of 35°C to 99.9°C
- sample block temperature uniformity of ≤0.75°C over the range of 55°C to 95°C
- temperature calibration traceable to a reference standard (i.e., NIST)

Program the thermocycler using the PCR Cycling Parameters specified below.

For specific thermocycler information refer to the manufacturer's user manual. Thermocyclers should be calibrated according to ASHI (American Society of Histocompatibility and Immunogenetics) or EFI (European Federation of Immunogenetics) accreditation rules.

Program the thermocycler before starting the Directions for Use described below.

104.101-12 – including Taq *polymerase*

For one KIR Genotyping typing, begin by adding to well No. 30, 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:

34 x 2 µl = 68 µl DNA (30 ng/µl)
34 x 3 µl = 102 µl PCR Master Mix complete with *Taq* – mix well before taking your aliquot
34 x 5 µl = 170 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-H_2O mixture into each of the 29 wells of an KIR Genotyping typing, i.e. wells 1 to 29. 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:

34 x 3 µl = 102 µl PCR Master Mix without *Taq* – mix well before taking your aliquot
2.7 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. 30, i.e. the well with the negative control primer pairs. Then add 7 µl dH_2O to well 30.

Then add at room temperature to the 0.5 ml tube containing 102 + 2.7 - 3 = 101,7 µl PCR Master Mix-*Taq* mixture:

34 x 2 µl = 68 µl DNA (30 ng/µl)
34 x 5 µl – 2.5 µl = 167.5+5 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-*Taq*-H_2O mixture into each of the 29 wells of an KIR Genotyping typing, i.e. wells 1 to 29. 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
4. End - hold	RT		if less than 8 hours
	4°C		if longer than 8 hours

Total reaction volume in each well, 10 µl.

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 carcinogen. Handle with appropriate personal protective equipment.**

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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***.

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 29+1 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	**800 bp**	2DL1 2DS1	0010101-068 013
2[4,7,9]	65 bp 150 bp 225 bp	1070 bp	2DL2 2DL2 2DL2	004, 011 0010101-015 004, 011
3[7]	520 bp	1070 bp	2DL3	0010101-011, 013-026, 028-036
4	200 bp	1070 bp	2DL4	00101-058
5[6]	140 bp	1070 bp	2DL5A 2DL5B	0010101-0010902, 0050101-0050105, 01201-01202, 014-015, 0210101-0340102, 040 0020101-0020104, 0020106-004, 00601-011, 01301-01304, 016-020, 035-0390102, 041
6[5, 8]	1650 bp	**430 bp**	2DL5A	0010101-0010902, 0050101-0050105, 01201-01202, 014-015, 0210101-0340102, 040
7[5,6,7,8]	1650 bp	**515 bp**	2DL5B	0020101-0020104, 0020106-004, 00601-011, 01301-01304, 016-020, 035-0390102, 041
8[4,11]	105 bp	1070 bp	2DS1	001-006, 008-012
9	205 bp	1070 bp	2DS2	0010101-022
10	140 bp	1070 bp	2DS3	00101-024
11	215 bp	1070 bp	2DS4	0010101-00106, 01101-01102, 014-017, 019-022
12	200 bp	1070 bp	2DS4	0030101-0040102, 0060101-010, 012, 013, 018
13[4,7]	110 bp	1070 bp	2DS5	001-038

14	90 bp	1070 bp	3DL1	0010101-0020105, 0050101-0090104, 0150101-018, 0200101-0200103, 022-03502, 038, 040-044, 051-054, 057[?], 05901-062, 064-068, 070, 071, 073-077, 079-081N, 086-090, 092-103, 109, 111-116, 118-1190102
	135 bp		3DL1	0010101-0020105, 0040101-0090104, 0150101-044, 051-054, 056, 057, 05901-077, 079-081N, 086-103, 109-1190102
15	200 bp	1070 bp	3DL2	0010101-116
16[4]	115 bp	1070 bp	3DL3	0010101-023, 02501-114
17	130 bp	1070 bp	3DS1	010-014, 045-050, 055, 058, 078, 082-085, 104-108
18	165 bp	1070 bp	2DP1	00101-004, 006-024
19[4]	125 bp	1070 bp	3DP1	001-052
20	235 bp	1070 bp	3DP1	0030101-00312, 005-00605, 008, 01001-1005, 013-052
21	145 bp	1070 bp	2DS1	001
22[4,10]	95 bp	1070 bp	2DS1	0020101-006, 008-012
	235 bp		3DP1	001-002, 004, 007, 0090101-00902, 011-012
23	210 bp	1070 bp	3DL1	0040101-00404, 019, 021, 036, 037, 039, 056, 072, 091, 110, 117
24[4,7,8]	100 bp	1070 bp	2DL4	00101-00604, 010, 01201-01202, 014-016, 018, 021-026, 028-034, 036-042, 045, 049, 050, 054, 056, 058
25	195 bp	1070 bp	2DL5B	0020101-0020104, 0020107-0020111, 00203[?]-00204[?], 00205, 004, 00601, 00603, 0070101, 0080101-00802, 00803[?]-00805[?], 009-011, 01301-01302, 01304[?], 016[?]-020[?], 035-0390102, 041[?]
			3DP1	001, 002[?], 007[?], 0090101-0090103, 00902[?], 01002[?], 011[?]-013[?]
26	160 bp	1070 bp	2DL5A	0010101-0010902, 0050101, 0050103-0050105, 01201-01202, 014[?]-015[?], 0210101-0210102, 022[?], 0230101-0230102, 024[?], 025-0340102, 040[?]
			2DS5	001[?], 0020101-0020133, 00202[?], 00203-0020702, 00202[?], 00203-

			3DP1	0020702, 003?-00501?, 00801?-009?, 011?-017?, 018-037, 038? 004
27	180 bp	1070 bp	2DL2	004
28[7]	130 bp	1070 bp	2DL3	0010101-0020103, 004 -015, 017-036
29	205 bp	1070 bp	2DS1 2DP1	001-00202, 004, 006, 008-013 006, 009, 010
30[6,12]	-	-	-	**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 KIR SSP typings.
When the primers in a primer mix can give rise to KIR-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits, the alleles listed are specified according to amplicon length.
Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.
PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.
PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.
Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an 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 internal positive control bands are 1070, 800, 515 or 430 base pairs respectively, well distribution as outlined in the table. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

[3]KIR alleles listed on the IPD KIR web page 2021-December-16, release 2.11.0, www.ebi.ac.uk/ipd/kir.

[4]Specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

[5]The specific PCR product generated by primer mixes 6 and 7 are longer than the internal positive control band and the positive control band may be weaker than for other KIR primer mixes.

[6]Primer mixes 5, 7 and 30 have a tendency to giving rise to primer oligomer formation.

[7]Primer mixes 2, 3, 7, 13, 24 and 28 may have tendencies of unspecific amplifications, most pronounced for primer mix 24.

[8]Primer mixes 6, 7 and 24 may give rise to a lower yield of specific PCR product than the other KIR primer mixes, most pronounced for primer mix 7.

[9]The 2DL2*004 and 2DL2*011 and the 2DL2*0010101-010 and 012-013 alleles may be distinguished by the different sizes of the specific PCR product in primer mix 2; three specific PCR fragments of 65, 150 and 225 bp in the 2DL2*004 and 2DL2*011 alleles and one specific PCR fragment of 150 bp in the 2DL2*0010101-00304, 005-010 and 012-013 alleles.

[10]The 2DS1 and the 3DP1 amplicons in primer mix 22 may be distinguished by the different sizes of the specific PCR product; a specific PCR fragment of 95 bp for the 2DS1*0020101-006 and 008-012 alleles and a specific PCR fragment of 235 bp for the 3DP1*001-002, 004, 007, 0090101-00902 and 011-012 alleles.

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[11]Primer mix 8 does not amplify the 2DS1*013 allele. Due to sequence homology between allele groups this allele is amplified in primer mixes 1 and 29. Hence, a sample that is positive for 2DL1 and 2DP1*006, 009 or 010 may be falsely interpreted as 2DS1-positive. 2DS1*013 is a rare, unconfirmed allele. Caution should be used when interpreting these results.

[12]Well 30 contains negative control primer pairs, that will produce exon 4 and/or exon 5 amplicons for the majority of applicable KIR alleles as well as amplicons generated by positive control primer pairs.

Abbreviations

'?', nucleotide sequence information not available for the primer matching sequence.

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Length of spec.	145	65	520	200	140	1650	1650	105	205	140	215	200	110	90	200
PCR product		150												135	
		225													
Length of int.	800	1070	1070	1070	1070	430	515	1070	1070	1070	1070	1070	1070	1070	1070
pos. control[1]															
5'-primer(s)[2]	130	156	332	208	226	-16	-16	165	140	236	229	234	142	136	110
	5'-gAA3'	5'-AAA3'	5'-TCg3'	5'-CCg3'	5'-CCA3'	5'-TCA3'	5'-TCg3'	5'-gAg3'	5'-gTA3'	5'-CAC3'	5'-CTA3'	5'-TCT3'	5'-ACC3'	5'-CAA3'	5'-ggg3'
	130	208	344				-16	165		236				336	
	5'-TAA3'	5'-CCA3'	5'-CTg3'				5'-Tgg3'	5'-gAA3'		5'-CAg3'				5'-TCC3'	
		262	344												
		5'-ggA3'	5'-CTg3'												
3'-primer(s)[3]	165	195	350	262	276	27	27	185	195	266	288	288	165	166	164
	5'-gCg3'	5'-ATg3'	5'-CAA3'	5'-ggA3'	5'-gAg3'	5'-ACA3'	5'-ACA3'	5'-gAC3'	5'-ATg3'	5'-CCT3'	5'-ggA3'	5'-ggA3'	5'-gTg3'	5'-CAA3'	5'-CAA3'
		243	351						195					343	
		5'-ACA3'	5'-ACC3'						5'-TAg3'					5'-AgA3'	
		269													
		5'-TAC3'													
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Well No.	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Length of spec.	115	130	165	125	235	145	95	210	100	195	160	180	130	205
PCR product							235							
Length of int.	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070
pos. control[1]														
5'-primer(s)[2]	156	133	29	25	2nd I	130	130	31	324	up[4]	up[5]	106	378	up[6]
	5'-CCC3'	5'-TCT3'	5'-CAT3'	5'-Tgg3'	5'-gCC3'	5'-gAA3'	5'-gAA3'	5'-TCA3'	5'-gTA3'	5'-AAg3'	5'-CCg3'	5'-CAC3'	5'-TAT3'	5'-CAA3'
							2nd I	31						
							5'-TCC3'	5'-TCA3'						
3'-primer(s)[3]	181	163	71	54	54	165	54	86	344	1st I	-16	165	405	-18
	5'-gTA3'	5'-ggA3'	5'-TAC3'	5'-TAC3'	5'-TAC3'	5'-gCC3'	5'-TAC3'	5'-CCA3'	5'-Tgg3'	5'-TCA3'	5'-gAT3'	5'-gCg3'	5'-CgA3'	5'-AgA3'
							165							
							5'-gCT3'							
Well No.	16	17	18	19	20	21	22	23	24	25	26	27	28	29

[1]The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 1070, 800, 515 or 430 base pairs respectively, well distribution as outlined in the table. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

[2]The codon position matching the specificity-determining 3'-end of the primer is given. Codon numbering as on the KIR web page 2018-November-30, release 2.8.0, www.ebi.ac.uk/ipd/kir. The sequence of the 3 terminal nucleotides of the primer is given.

[3]The codon position matching the specificity-determining 3'-end of the primer. Codon numbering as on the KIR web page 2021-December-16, release 2.11.0, www.ebi.ac.uk/ipd/kir. The sequence of the 3 terminal nucleotides of the primer is given in the anti-sense direction.

[4]Primer located upstream of the 1st exon, 84 nucleotides upstream of codon -21.

[5]Primer located upstream of the 1st exon, 104 nucleotides upstream of codon -21.

[6]Primer located upstream of the 1st exon, 154 nucleotides upstream of codon -21.

Lot No.: 7N4 **Lot-specific information**

CELL LINE VALIDATION SHEET

KIR Genotyping primer set[2]

			Well															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		Prod. No.:	202237501	202237502	202237503	202237504	202237505	202237506	202237507	202237508	202237509	202237510	202237511	202237512	202237513	202237514	202237515	202237516
	IHWC cell line[1]																	
1	9001	SA	+	-	+	+	-	-	-	-	-	-	+	-	-	+	+	+
2	9280	LK707	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+
3	9011	E4181324	+	-	+	+	-	-	-	-	-	-	+	+	-	+	+	+
4	9275	GU373	+	-	+	+	-	-	-	-	-	-	+	+	-	+	+	+
5	9009	KAS011	+	-	+	+	+	+	-	+	-	-	-	+	+	+	+	+
6	9353	SM	+	-	+	+	+	+	-	+	-	+	+	-	-	+	+	+
7	9020	QBL	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
8	9025	DEU	+	+	+	+	-	-	-	-	+	-	-	+	-	+	+	+
9	9026	YAR	+	-	+	+	-	-	-	-	-	-	+	+	-	+	+	+
10	9107	LKT3	+	-	+	+	-	-	-	-	-	-	+	-	-	+	+	+
11	9051	PITOUT	+	+	+	+	-	-	-	-	+	-	-	+	-	+	+	+
12	9052	DBB	+	+	+	+	+	-	+	-	+	+	-	+	-	+	+	+
13	9025	JESTHOM	+	+	-	+	+	-	+	-	+	+	+	+	-	+	+	+
14	9071	OLGA	+	-	+	+	+	+	-	+	-	-	-	+	+	+	+	+
15	9075	DKB	+	-	+	+	-	-	-	-	-	-	+	-	-	+	+	+
16	9037	SWEIG007	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+
17	9282	CTM3953540	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
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	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+

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Lot No.: 7N4 **Lot-specific information**

CELL LINE VALIDATION SHEET

KIR Genotyping primer set[2]

			Well												
			17	18	19	20	21	22	23	24	25	26	27	28	29
		Prod. No.:	202237517	202237518	202237519	202237520	202237521	202237522	202237523	202237524	202237525	202237526	202237527	202237528	202237529
	IHWC cell line[1]														
1	9001	SA	-	+	+	+	-	-	-	+	-	-	-	+	-
2	9280	LK707	-	-	+	-	-	+	-	-	+	+	-	-	+
3	9011	E4181324	+	+	+	+	-	+	+	+	-	+	-	+	-
4	9275	GU373	-	+	+	+	-	-	-	+	-	-	-	+	-
5	9009	KAS011	+	+	+	+	-	+	-	+	-	+	-	+	+
6	9353	SM	+	+	+	+	-	+	-	+	-	+	-	+	+
7	9020	QBL	+	+	+	+	-	+	+	+	+	+	-	+	+
8	9025	DEU	-	+	+	+	-	+	-	-	+	-	-	+	-
9	9026	YAR	-	+	+	+	-	-	-	+	-	-	-	+	-
10	9107	LKT3	-	+	+	+	-	-	-	+	-	-	-	+	-
11	9051	PITOUT	-	+	+	+	-	+	+	-	+	-	-	+	-
12	9052	DBB	-	+	+	+	-	-	+	-	+	-	-	+	-
13	9025	JESTHOM	-	+	+	+	-	+	-	+	+	-	-	-	-
14	9071	OLGA	+	+	+	+	-	+	-	+	-	+	-	+	+
15	9075	DKB	-	+	+	+	-	-	-	+	-	-	-	+	-
16	9037	SWEIG007	-	+	+	+	-	-	+	-	-	-	-	+	-
17	9282	CTM3953540	+	+	+	+	-	+	+	+	+	+	-	+	+
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	-	+	+	+	-	-	-	-	-	-	-	+	-

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[1]The provided cell line KIR specificities are retrieved from the www.ebi.ac.uk/ipd/kir web site. The specificity of an individual cell line may thus be subject to change.

[2]The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs.
No DNAs carrying the alleles to be amplified by primer solution 21 were available.
One additional primer in primer solution 3 was tested by separately adding another 5'-primer.
In primer solution 21 it was only possible to test the 5'-primer, the 3'-primer was not possible to test.
In primer solutions 1, 2, 3, 7, 10 and 16 one of the 5'-primers were not possible to test, and in primer mix 2, 9, 16 and 24 one 3'-primer was not possible to test.

TRADEMARKS USED IN THIS DOCUMENT/PRODUCT

Olerup SSP® is a registered trademark of *CareDx* AB.
Qiagen™ is a trademark of QIAGEN.

WARRANTY

CareDx AB warrants its products to the original purchaser against defects in materials and workmanship under normal use and application. *CareDx* 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 *CareDx* 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 *CareDx* 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 *CareDx* 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 *CareDx* AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.
Handle all samples as if capable of transmitting disease. All work should be performed wearing gloves and appropriate protection.

GUARANTEE

CareDx 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.
When stored at –20ºC, the dried primers are stable for 48 months from the date of manufacture.
When stored at –20ºC, the PCR Master Mix including *Taq* polymerase and the PCR Master Mix without *Taq* polymerase are stable for 51 months from the date of manufacture.

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