104.201-12 – including *Taq* polymerase
104.201-12u – without *Taq* polymerase
Visit www.caredx.com for **"Instructions for Use" (IFU)**
Lot No.: 5S6 **Lot-specific information**

Olerup SSP® KIR HLA Ligand

Product number:	**104.201-12 – including *Taq* polymerase** **104.201-12u – without *Taq* polymerase**
Lot number:	**5S6**
Expiry date:	**2027-10-01**
Number of tests:	**12**
Number of wells per test:	**7+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. 5S6.

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 HLA LIGAND LOT (8N3)

- The product documentation has been updated for new alleles of IMGT 3.53.0.

The KIR HLA Ligand specificity and interpretation tables have been updated for the HLA-A, HLA-B and HLA-C alleles described since the previous *Olerup* SSP® KIR HLA Ligand lot was made **(Lot No. 8N3)**.

The KIR HLA Ligand primer set is unchanged compared to the previous *Olerup* KIR HLA Ligand lot **(Lot No. 8N3)**.

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Lot No.: 5S6 **Lot-specific information**

Well **8** contains Negative Control primer pairs, that will amplify the majority of the *Olerup* SSP® HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

HLA-specific PCR product sizes range from 75 to 200 base pairs.
The PCR product generated by the positive control primer pair is 200 base pairs.

Length of PCR product	**105**	**200**	**105**	**80**	**75**	**80**	**85**
5'-primer[1]	**164**	**340**	**440**	**45**	**45**	**43**	**36**
	5'-CAC3'	**5'-Agg3'**	**5'-TTA3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-TAC3'**
							36
							5'-TAT3'
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**	**47**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**	**5'-ACA3'**
							48
							5'-gCA3'
							48
							5'-gCC3'
							52
							5'-TgT3'
A*	**+**	**+**	**+**				
B*	**+**	**+**	**+**				
C*	**+**	**+**	**+**				
DRB1				**+**	**+**		
DRB3				**+**	**+**		
DRB5				**+**			
DQB1					**+**		
DPB1						**+**	
DQA1							**+**

[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 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.
[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.

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104.201-12u – without *Taq* polymerase
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Lot No.: 5S6 **Lot-specific information**

PRODUCT DESCRIPTION

KIR HLA Ligand SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for determining KIR HLA Ligand nucleotide sequence motifs;
HLA-A^{Bw4+} alleles,
HLA-B^{Bw4+} alleles encoding Isoleucine or Threonine at position 80,
HLA-B^{Bw4+} alleles encoding Aspartic acid at position 77 and Threonine at position 80,
HLA-B^{Bw6+} alleles encoding Asparagine at position 80,
HLA-C alleles encoding Asparagine or Lysine at position 80.

PLATE LAYOUT

Each test consists of 8 PCR reactions in an 8 well cut PCR plate.

1	2	3	4	5	6	7	NC

The 8 well cut PCR plate is marked with '5S6' in silver/gray ink.
Well No. 1 is marked with the Lot Number '5S6'.
Wells 1 and 2: HLA-C KIR ligand primers.
Wells 3, 4, 6 and 7: HLA-B KIR ligand primers.
Well 5: HLA-A KIR ligand primers.
Well 8 – Negative Control (NC).
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 8 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

The HLA-A, HLA-B and HLA-C alleles recognized by the HLA Nomenclature Committee in July 2023[1,2,3] have been considered in the Specificity and Interpretation Tables.

[1]A minor subset of rare B and C alleles with the listed target motifs will not be amplified by the kit due to downstream sequence variations.
[2]HLA-A, HLA-B and HLA-C alleles listed on the IMGT/HLA web page 2023-July-12, release 3.53.0, www.ebi.ac.uk/imgt/hla.
[3]Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page http://hla.alleles.org/alleles/deleted.html.

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Lot No.: 5S6 **Lot-specific information**

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

104.201-12 – including *Taq* polymerase

For one KIR HLA Ligand typing, begin by adding to well 8, 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:

9 x 2 µl = 18 µl DNA (30 ng/µl)
9 x 3 µl = 27 µl PCR Master Mix with *Taq* – mix well before taking your aliquot
9 x 5 µl = 45 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-H_2O mixture into each of the 7 wells of a KIR HLA Ligand typing, i.e. wells 1 to 7. 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.

104.201-12u – without *Taq* polymerase

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

10 x 3 µl = 30 µl PCR Master Mix without *Taq* – mix well before taking your aliquot
0,8 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. 8, i.e. the well with the negative control primer pairs. Then add 7 µl dH_2O to well 8.

Then add at room temperature to the 0.5 ml tube containing 30 + 0,8 - 3 = 27,8 µl PCR Master Mix-*Taq* mixture:

9 x 2 µl = 18 µl DNA (30 ng/µl)
9 x 5 µl – 0.8 µl = 44.2 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-*Taq*-H_2O mixture into each of the 7 wells of a KIR HLA Ligand typing, i.e. wells 1 to 7. 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 bottle (Product No. 103.301-10), 1 drop of ethidium bromide solution per 50-75 ml of gel, or our GelRed™ dropper bottle (Product No. 103.302-05) 4 drops per 100-120 ml of gel solution. **Note: Ethidium bromide is a powerful carcinogen. Handle with appropriate personal protective equipment.**

Load the PCR products, preferably using an 8-channel pipette. Load a DNA size marker (100 base pair ladder, Product No. 103.202-100 or DNA Size Marker for short gel runs 103.203-100) 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.

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104.201-12u – without *Taq* polymerase
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Lot No.: 5S6 **Lot-specific information**

SPECIFICITY TABLE

KIR HLA Ligand SSP typing

Specificities and sizes of the PCR products of the 7+1 primer mixes used for KIR HLA Ligand SSP.

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	KIR HLA Ligand nucleotide sequence motif	Amplified HLA alleles[3,4]
1[7]	345 bp	**800 bp**	HLA-C^{Asn80}	C*01:02:01:01-01:02:50, 01:02:52-01:02:88, 01:02:90-01:13, 01:15:01-01:45, 01:47-01:153, 01:155-01:170, 01:172-01:253, 02:27:01-02:27:02, 02:65, 02:87, 02:115, 02:131, 03:02:01-03:04:94, 03:04:96-03:06:02, 03:08-03:09, 03:11:01-03:11:02, 03:13:01:01-03:14, 03:16-03:28, 03:30-03:44, 03:46-03:98, 03:100-03:162, 03:164-03:267, 03:269-03:296:02, 03:298-03:394, 03:396:01N, 03:397-03:398, 03:400-03:414, 03:416-03:437, 03:439-03:449N, 03:451-03:460, 03:462N-03:500, 03:502Q-03:626, 03:628N-03:640, 04:11, 04:29, 04:36, 04:55, 04:114, 04:172, 04:346, 04:383, 04:473, 05:20, 06:11, 06:82, 06:147, 06:210, 06:217, 06:248, 06:252, 07:01:01:01-07:01:02:15, 07:01:04-07:06:06, 07:08, 07:10-07:33N, 07:35-07:75, 07:77-07:78:01, 07:79-07:114, 07:116-07:294, 07:296-07:314:03, 07:316-07:327, 07:329N-07:360, 07:362-07:405:02, 07:407-07:425, 07:427-07:458, 07:460-07:477, 07:479-07:558:01:02, 07:560-07:575:02, 07:577-07:597, 07:599-07:655, 07:657-07:672N, 07:674-07:715:02, 07:717-07:913, 07:915-07:950, 07:952-07:1052, 07:1054N-07:1082, 08:01:01:01-08:01:07, 08:01:09-08:09, 08:11-08:63, 08:65-08:145, 08:147-08:270, 12:02:01-12:02:22, 12:02:24-12:03:69, 12:03:71-12:03:83, 12:06-12:08, 12:10:01-12:20, 12:22-12:32, 12:34, 12:36-12:40, 12:42Q-12:59, 12:61-12:71, 12:73-12:134, 12:136-12:145, 12:147-12:153, 12:155Q-12:229, 12:231-12:298, 12:300-12:352, 12:354-12:389, 14:02:01:01-14:11, 14:13-14:48, 14:50-14:107, 14:109-14:154, 15:07:01:01-15:07:01:02, 15:25, 15:43, 15:85, 15:144, 15:181, 16:01:01:01-16:01:43, 16:04:01:01-16:04:01:03, 16:04:03-16:04:05, 16:06-16:08, 16:10-16:11, 16:13-16:18, 16:20-16:24, 16:26-16:36, 16:38-16:45, 16:49:01-16:59, 16:61-16:62, 16:64-16:68, 16:71-16:73, 16:75-16:76, 16:78-16:83, 16:86-16:87, 16:92-16:93, 16:95-16:98, 16:100,

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2^8	350 bp	**800 bp**	HLA-C^{Lys80}	C*01:14, 01:154, 02:02:01-02:02:03, 02:02:06-02:26:03, 02:28-02:40:02, 02:42-02:64, 02:66-02:86, 02:88-02:114, 02:116-02:130, 02:132-02:186, 02:188-02:223N, 03:07:01:01-03:07:02, 03:10, 03:15, 03:29, 03:45, 03:163, 03:268, 03:297, 03:450, 03:461, 04:01:01:01-04:01:01:29, 04:01:01:31-04:01:131, 04:01:133-04:01:153, 04:03:01:01-04:10, 04:12-04:20, 04:23-04:28, 04:30-04:35:02, 04:37-04:54:02, 04:56-04:113, 04:115N-04:171, 04:173N-04:249, 04:251-04:345, 04:347-04:382Q, 04:384-04:472, 04:474-04:507, 05:01:01:01-05:01:75, 05:03-05:19, 05:21-05:244N, 05:246-05:285, 06:02:01:01-06:02:01:94, 06:02:03-06:02:86, 06:02:88-06:02:90, 06:02:92-06:10, 06:12-06:81, 06:83-06:146, 06:148-06:209:02, 06:211:01:01N-06:216, 06:218-06:247, 06:249-06:251, 06:253-06:364, 07:07, 07:09, 07:76:01-07:76:02, 07:315, 07:328, 07:406, 07:559, 07:598, 07:656, 07:914, 08:10, 12:04:01-12:05:02, 12:09, 12:21, 12:33, 12:41, 12:60, 12:72, 12:135, 12:146, 12:154, 12:353, 14:12, 14:49, 14:108, 15:02:01:01-15:05:07, 15:05:09-15:06:03, 15:08:01-15:13:02, 15:15-15:19, 15:21-15:24, 15:26-15:42, 15:44:01-15:84Q, 15:86-15:143, 15:145N-15:180, 15:182-15:260, 16:02:01:01-16:02:20, 16:09, 16:12, 16:19, 16:25, 16:37, 16:46-16:48, 16:60, 16:63, 16:69-16:70, 16:74, 16:77N, 16:84, 16:88-16:91, 16:99, 16:101-16:104, 16:107-16:108, 16:115, 16:120-16:121, 16:123N, 16:132N-16:133, 16:136, 16:140, 16:143-16:145, 16:153, 16:155-16:156, 16:163, 16:166-16:167, 16:176, 16:179, 16:181, 16:184, 16:194-16:195N, 16:202, 17:01:01:02-17:21, 17:23-17:71, 18:01:01:01-18:19
3^5	350 bp	**800 bp**	HLA-$B^{Bw4+Thr80}$	B*07:149, 07:453, 08:02, 08:117, 08:255, 08:271, 13:01:01:01-13:04, 13:06-13:08, 13:10-13:23, 13:25-13:38, 13:40-13:66, 13:68-13:180, 14:118, 15:36, 15:89:01-15:89:02, 15:115, 15:256, 15:339, 15:505, 18:09, 27:01, 27:142, 37:01:02, 37:10, 38:02:01:01-38:04, 38:08, 38:15, 38:18, 38:23, 38:29, 38:35, 38:43-38:50, 38:62, 38:64, 38:72, 38:74-38:76, 38:79, 38:82,

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4[5]	350 bp	1070 bp	HLA-B$^{Bw4+Ile80}$	B*07:36, 07:38, 07:81, 07:180, 07:219, 08:03, 08:52, 08:78, 14:93, 15:13:01-15:13:03, 15:16:01:01-15:17:07, 15:23-15:24:02, 15:67, 15:87, 15:95, 15:157, 15:162, 15:168, 15:177, 15:196, 15:208, 15:216, 15:222, 15:230, 15:254, 15:268, 15:273, 15:356, 15:361-15:362, 15:396, 15:403, 15:408, 15:411, 15:418, 15:423-15:424, 15:442, 15:446, 15:462, 15:500, 15:516, 15:523, 15:532, 15:546Q, 15:550, 15:555, 15:575N, 15:586, 15:602-15:603, 15:613, 15:619, 15:642, 15:644, 15:655, 18:67, 18:136, 18:194:01:01-18:194:01:02, 27:02:01:01-27:02:06, 27:30, 27:53, 27:57, 27:62, 27:65N, 27:75, 27:77, 27:83, 27:95, 27:102, 27:119, 27:126, 27:134, 27:157, 27:163, 27:171-27:172, 27:176N, 27:181, 27:188, 27:197, 27:203, 27:213, 27:232, 27:236, 27:245-27:246N, 27:251, 35:471, 37:34, 38:01:01:01-38:01:22, 38:05-38:07, 38:09-38:14, 38:16, 38:19-38:22, 38:24-38:28, 38:30-38:34N, 38:36-38:42, 38:51-38:61, 38:63, 38:65-38:71, 38:73, 38:77-38:78, 38:80N-38:81, 38:83N, 38:86-38:88, 38:90-38:91, 38:93-38:96, 38:99-38:102, 38:106-38:111, 38:161, 38:166, 38:168-38:171, 40:13, 40:19, 40:109, 40:117, 40:292, 40:340, 40:394, 41:46, 41:60, 44:06, 44:18:01:01-44:18:01:02, 44:25, 44:50:01, 44:50:03, 44:95, 44:355, 48:18, 49:01:01:01-49:01:24, 49:03-49:14, 49:16-49:69, 49:71-49:81, 51:01:01:01-51:24:05, 51:26-51:46, 51:48-51:53, 51:55-51:77, 51:79-51:340, 51:342-51:370, 51:372-51:386, 52:01:01:01-52:01:28, 52:01:30-52:19, 52:21:01-52:115N, 53:01:01:01-53:02, 53:04-53:08:02, 53:10, 53:14-53:30, 53:32-53:35, 53:37, 53:39-53:54,

				53:56-53:66, 53:68-53:73, 54:12, 55:103, 56:21, 56:65, 57:01:01:01-57:11, 57:13-57:167, 58:01:01:01-58:02:02, 58:04-58:16:02, 58:18-58:29, 58:31N-58:63, 58:65-58:136, 58:138-58:143, 59:01:01:01-59:07, 59:09-59:12
5	370 bp	1070 bp	HLA-A^{Bw4+}	A*01:95, 01:289, 01:298, 02:81, 02:87, 02:112, 02:124, 02:129, 02:136, 02:571, 02:829, 03:152, 03:219, 11:178, 11:190, 23:01:01:01-23:68, 23:70-23:89, 23:91N-23:111, 23:114-23:121, 23:123-23:131, 24:02:01:01-24:02:163, 24:02:165-24:03:04, 24:05:01-24:11N, 24:13:01-24:15, 24:17:01:01-24:18, 24:20:01:01-24:27, 24:29-24:43, 24:45N-24:64, 24:66-24:88, 24:90:01N-24:99, 24:101-24:108, 24:110-24:128, 24:130-24:210, 24:212-24:240N, 24:242-24:289, 24:291-24:372, 24:374-24:405, 24:407-24:423, 24:425N-24:470, 24:472-24:608N, 25:01:01:01-25:58, 25:60-25:86, 29:13, 31:07-31:08, 31:10, 31:222, 32:01:01:01-32:175, 68:36, 68:249
6	350 bp	1070 bp	HLA-B$^{Bw4+,\ Thr80}$	B*07:27, 07:236, 07:273, 08:126, 15:43, 15:594, 18:54, 18:195, 27:03-27:05:36, 27:05:38-27:05:58, 27:06:01:01-27:07:06, 27:09-27:11, 27:13:01-27:17, 27:19:01:01-27:21:02, 27:23-27:25, 27:27-27:29, 27:31-27:32, 27:34-27:39, 27:41, 27:43, 27:45-27:48, 27:50:01-27:52, 27:54-27:56, 27:58-27:61, 27:63-27:64N, 27:66N-27:74, 27:76, 27:78-27:82, 27:84-27:88, 27:90:01-27:94N, 27:96:01-27:101, 27:103-27:118, 27:120-27:125, 27:127-27:133, 27:135-27:141, 27:143-27:152, 27:154-27:155, 27:158-27:162, 27:164, 27:166-27:170, 27:173-27:175, 27:177-27:180, 27:182-27:187, 27:189-27:196, 27:198-27:202, 27:205-27:212N, 27:214, 27:216-27:231, 27:233-27:235, 27:237-27:241, 27:243N-27:244, 27:247-27:250, 27:252-27:259, 27:261N-27:267, 35:329, 37:01:01:01-37:01:01:19, 37:01:03-37:04:02, 37:06:01-37:09, 37:12-37:13, 37:15-37:33N, 37:35-37:36, 37:38-37:66, 37:68-37:99, 37:101-37:108, 38:17, 40:188, 44:257, 47:01:01:02-47:01:05, 47:05-47:10, 47:12-47:13, 53:03, 53:55, 55:90, 55:121, 56:46
7	350 bp	1070 bp	HLA-B^{Bw6+}	B*07:02:01:01-07:02:05, 07:02:07-07:02:77, 07:02:79-07:26, 07:28-07:35, 07:37:01-07:37:02, 07:39-07:80, 07:82-07:148, 07:150-07:179, 07:181N-07:191, 07:193-07:218, 07:220-07:235, 07:237-07:272N, 07:274-07:452, 07:454-07:483, 08:01:01:01-08:01:71, 08:04:01-08:05, 08:07-08:51, 08:53:01-08:77, 08:79:01-08:116, 08:118-08:125, 08:127-08:130, 08:132-08:135, 08:137-08:138,

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8[6]	-	-	**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 HLA Ligand SSP typings.
When the primers in a primer mix can give rise to HLA-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 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 bp, internal positive control band. 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]For several HLA Class I alleles 1st and/or 4th exon(s) and beyond, as well as intron nucleotide sequences, are not available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

[4]A minor subset of rare B and C alleles with the listed target motifs will not be amplified by the kit due to downstream sequence variations.

[5]Primer mixes 3 and 4 may have tendencies of unspecific amplifications.

[6]Primer mix 8 contains a negative control, which will amplify the majority of HLA amplicons as well as the amplicons generated by the control primer pairs matching the human growth hormone gene. HLA-specific PCR product sizes range from 75 to 200 base pairs and the PCR product generated by the HGH positive control primer pair is 200 base pairs.

[7]This lot will not amplify the C*01:46 allele in primer mix 1.

[8]The alleles C*05:32 (80^{Arg}), C*15:60 (80^{Gln}) and C*15:71(80^{Ile}) will be amplified in primer mix 2.

Abbreviations
w: might be weakly amplified.
Asn: asparagine
Asp: aspartic acid
Ile: isoleucine
Lys: lysine
Thr: threonine

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7
Length of spec. PCR product	345	350	350	350	370	350	350
Length of int. pos. control[1]	800	800	800	1070	1070	1070	1070
5'-primer(s)[2]	1st I	1st I	1st I	1st I	1st I	1st I	1st I
	5'-CgA 3'	5'-CgA 3'	5'-CAg 3'	5'-CAg 3'	5'-gCA 3'	5'-CAg 3'	5'-CAg 3'
3'-primer(s)[3]	310	312	309	309	317	310	311
	5'-gTT 3'	5'-AgT 3'	5'-gTg 3'	5'-ATC 3'	5'-ggA 3'	5'-ggT 3'	5'-ggT 3'
	311	312	312	312			
	5'-gAT 3'	5'-AgT 3'	5'-gCC 3'	5'-gCA 3'			
	311	312	313				
	5'-ggT 3'	5'-AgT 3'	5'-Cgg 3'				
	316		315				
	5'-gCT 3'		5'-AgT 3'				
Well No.	1	2	3	4	5	6	7

[1]The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 bp, internal positive control band. 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 nucleotide position 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 matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

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CELL LINE VALIDATION SHEET

KIR HLA Ligand primer set[2]

			Well						
			1	2	3	4	5	6	7
		Prod. No.:	202358101	202358102	202358103	202358104	202358105	202358106	202358107
	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	9004	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	+	+	+	-	-	-	+

[1]The provided cell line HLA specificities are retrieved from the http://www.ihwg.org/hla 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.
In primer solutions 1 to 4 one, two or three of the 3'-primers were not possible to be tested.

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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.

ADDRESSES:

Manufacturer:

Caredx AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.
Tel: +46-8-508 939 00
Fax: +46-8-717 88 18
E-mail: orders-se@caredx.com
Web page: www.caredx.com

CareDx Lab Solutions Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382
Tel: 1-877-653-78171
Fax: 610-344-7989
E-mail: orders-us@caredx.com
Web page: www.caredx.com

For information on CareDx distributors worldwide, contact **CareDx AB**.