Instructions for Use

Olerup SSP®
without Taq polymerase

For *In Vitro* Diagnostic Use

INTENDED USE

Olerup SSP® HLA Typing Kits are qualitative *in vitro* diagnostic kits for the DNA typing of HLA Class I and HLA Class II alleles. The products are used by trained professionals in medical settings for the purpose of determining HLA phenotype. The source material tested is DNA.

SUMMARY AND EXPLANATION

Human leukocyte antigens (HLA) used to be determined by the lymphocytotoxicity test. However, this test has been replaced by polymerase chain reaction (PCR)-based DNA typing techniques due to its error rate and lack of allele level resolution. In most PCR-based techniques, the PCR process is used only as an amplification step of needed target DNA and a post-amplification step to discriminate between the different alleles is required. In contrast, in the PCR-SSP methodology (sequence-specific primer – SSP), the discrimination between the different alleles takes place during the PCR process. This shortens and simplifies the post-amplification step to a simple gel electrophoresis detection step. The SSP test results are either positive or negative, which abolishes the need for complicated interpretation of results. In addition, the typing resolution of the PCR-SSP is higher than for other PCR-based typing techniques as each primer pair defines two sequence motifs located in *cis*, i.e. on the same chromosome. Furthermore, due to the synthetic nature of SSP reagents stability has been improved and lot to lot variation reduced.

PRINCIPLE OF PROCEDURE

The PCR-SSP methodology is based on the principle that completely or almost completely matched oligonucleotide primers without 3-'end mismatches are more efficiently used in the PCR reaction, than mismatched primers by thermo-stable DNA polymerases without proof-reading properties. Primer pairs are designed to be matched with single alleles or group(s) of alleles depending upon the degree of typing resolution required. With strictly controlled PCR conditions, matched or almost completely matched primer pairs allow amplification to occur, i.e. a positive result, whereas mismatched primer pairs don't allow amplification to occur, i.e. a negative result.

After the PCR process, the amplified DNA fragments are size-separated e.g. by agarose gel electrophoresis, visualized by staining with ethidium bromide and exposure to ultraviolet light, documented by photography and interpreted. Interpretation of PCR-SSP results is based on the presence or absence of specific PCR product(s). The relative sizes of the specific PCR product(s) may be helpful in the interpretation of the results. The PCR-SSP methodology for HLA was originally described by O. Olerup in 1991 and 1992[1,2].

Since the PCR process may be adversely affected by various factors (e.g. pipetting errors, too low DNA concentration, poor DNA quality, presence of PCR inhibitors, thermal cycler accuracy) an internal positive control primer pair is included in each PCR reaction[2]. The internal positive control primer pair matches conserved regions of the human growth hormone gene, which is present in all human DNA samples. In the presence of a specific PCR product of an HLA allele(s), the product of the internal positive control band may be weak or absent. The amplicons generated by the specific HLA primer pairs are shorter than the amplicons of the internal positive control primer pair but larger than unincorporated primers (see Expected values).

REAGENTS

A. Identification

The *Olerup* SSP® typing kits contain dried, pre-optimized sequence-specific primers for PCR amplification of HLA alleles and of the human growth hormone gene, PCR Master Mix without *Taq* polymerase ("Master Mix"), adhesive PCR seals and the basic Instructions for Use pamphlet.

The primer solutions are pre-aliquoted and dried in different 0.2 ml wells of cut, thin-walled PCR trays. Each well of the tray contains a dried primer solution consisting of a specific primer mix, i.e. allele- and group-specific HLA primers as well as an internal positive control primer pair matching non-allelic sequences and are ready for the addition of DNA sample, Master Mix, and H_2O.

The primers are designed for optimal PCR amplification when using the Master Mix and the recommended DNA cycling program (see Programming the Thermocycler).

Lot-specific Specificity and Interpretation Tables or Worksheet for the specific HLA alleles amplified by each primer mix can be retrieved from the webpage https://labproducts.caredx.com/products/olerup-ssp/.

B. Warnings and Precautions

1. For *In Vitro* Diagnostic Use.
2. This product cannot be used as the sole basis for making a clinical decision.
3. *Biohazard Warning*: All blood products should be treated as potentially infectious. No known test method(s) can offer assurance that products derived from human blood will not transmit infectious agents.
4. *Biohazard Warning*: The ethidium bromide used for staining DNA in the agarose gel electrophoresis is a carcinogen. Handle with appropriate personal protective equipment.
5. *Caution*: Wear UV-blocking eye protection, and do not view UV light source directly when viewing or photographing gels.
6. Pipettes and other equipment used for ***post***-PCR manipulations should ***not*** be used for ***pre***-PCR manipulations.

7. See Safety Data Sheet (http://www.labproducts.caredx.com) for detailed information.

C. Instructions for use

See Directions for Use.

D. Storage Instructions

Store kit components in the dark and at temperatures indicated on package labels.

Use before the Expiration Date printed on package labels.

E: Purification or Treatment Required for Use

See Directions for Use.

F. Instability Indications

1. Do not use primer trays with cracks in the wells or damage to the upper rim of the wells as this may cause evaporation during PCR amplification. Do not use PCR cap strips with cracks as this may cause evaporation during PCR amplification.
2. The pellets in the wells should be red in color. Yellow discoloration of pellet may indicate degradation.
3. Master Mix should be red to purple in color. Yellow to orange discoloration may indicate degradation.

INSTRUMENT REQUIREMENTS

A. Instrument

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 correspond 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 in Section B 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.

B. 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.

SPECIMEN COLLECTION AND PREPARATION

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 $A_{260/280}$ 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.

PROCEDURE

A. Materials provided

1. *Olerup* SSP® primer trays.
2. Master Mix without *Taq* polymerase (appropriate volume for the trays of the kit). The same Master Mix is used for all *Olerup* SSP® kits.
3. Adhesive PCR seals (appropriate number for the trays of the kit).
4. Basic Instructions for Use pamphlet.

B. Materials Required but not Provided

1. DNA isolation kit/equipment
2. UV spectrophotometer
3. Pipetting devices. We recommend electronic single-channel dispenser capable of dispensing 10 µl aliquots for adding the DNA-Master Mix-dH_2O mix to the tray wells.
4. Disposable pipette tips
5. Polypropylene tubes
6. Vortex mixer
7. Microcentrifuge
8. PCR tray rack
9. Thermocycler with heated lid for PCR with 96-well format, a temperature gradient across the heating block ≤ 0.75°C, and tray/retainer for 0.2 ml thin-walled reaction wells
10. Microwave oven or hot plate for heating agarose solutions
11. Electrophoresis grade agarose, e.g. FMC Seakem LE
12. 0.5 x TBE buffer; 1 x TBE buffer is 89 mM Tris-borate, 2 mM disodium EDTA, pH 8.0
13. Ethidium bromide dropper bottle, Product No. 103.301-10
14. Gel-loading pipetting device. We recommend 8 channel pipette for gel-loading, 5-25 µl adjustable volume
15. DNA size marker to cover range of 50 – 1 000 bp, e.g. 100 base pair ladder, DNA Size Marker Product No. 103.202-100 or DNA Size Marker for short gel runs 103.203-100
16. Electrophoresis apparatus/power supply
17. UV transilluminator
18. Photographic or image documentation system
19. Roche *Taq* DNA polymerase, GMP Grade. [Catalog # 03 734 927 001 (1000 U) or # 03 734 935 001 (5000 U)].

C. Step-by-step Procedure

See Directions for Use.

DIRECTIONS FOR USE

A. Sample preparation

1. Purify genomic DNA from leukocyte sample by method of choice, see Specimen Collection and Preparation above.
2. For specific information on sample preparation and storage, see Specimen Collection and Preparation above.
3. Perform PCR amplification on purified DNA sample using an *Olerup* SSP® typing tray, or store DNA sample until ready to type.

B. Reagent/Equipment Preparation

1. Program a thermocycler to run the *Olerup* SSP® PCR program, see Instrument Requirements – PCR Cycling Parameters above.
2. Have *Taq* polymerase available (5 units/µl), store at -20°C.
3. Prepare electrophoresis gel, see section C – **Gel Electrophoresis Preparation** below.

C. Gel Electrophoresis Preparation

For *Olerup* SSP® Gel System 96 (Product No. 103.101-01)

1. Set-up
   - Level the casting chamber for 1 gel (Product No. 103.101-31) or the casting chamber for 3 gels (Product No. 103.101-33) by using the leveling bubble and the three height-adjustable legs.
   - Place the gel tray(s) in the casting chamber.
2. 2% (w/v) Agarose Gel Preparation

   Use a high-quality electrophoresis grade agarose, capable of resolving 50 – 2 000 base pair fragments of DNA.
   - To 5 ml of 10 x TBE (Tris Borate EDTA) buffer add 150 ml of distilled water and 2 g of agarose in a 500 ml glass bottle.
   - Dissolve the agarose by boiling in a microwave oven until a homogenous solution of 100 ml is formed.
   - Let the dissolved gel solution cool to 60°C, e.g. in a heating cabinet.
   - 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). **Note: Ethidium bromide is a carcinogen. Handle with appropriate personal protective equipment.**
   - Pour 100 ml of gel solution into the gel tray in the casting chamber. Place 6 gel combs (Product No. 103.101-21) in the slots of the gel tray.
   - Allow the gel to set for 15 minutes.
   - Pour 750 ml of 0.5 x TBE buffer into the gel tank. Immerse the gel tray in the gel box and carefully remove the 6 gel combs by lifting them up.

Follow the manufacturer's instructions for use when using alternative electrophoresis systems. In order to be used with *Olerup* SSP® HLA Typing

Kits, these systems must be capable of resolving PCR products ranging from 50 to 1100 base pairs in size.

D. Stepwise Procedure

1. Remove from the indicated storage temperature(s): the appropriate number of DNA samples, the primer tray(s), the volume of Master Mix and *Taq* polymerase (5 units/µl) needed for the selected DNA sample(s)/primer tray(s). Thaw at room temperature (20 to 25°C)

The same Master Mix is used for all for all *Olerup* SSP® kits.

2. Mix DNA sample(s) briefly by vortexing.
3. Place the primer tray(s) in a PCR tray rack.
4. **Low and High resolution kits**
   - Vortex the Master Mix before taking aliquots.
   - Using a manual single-channel pipette, add at room temperature Master Mix, *Taq* polymerase (5 units/µl) and dH_2O into a 0.5 ml or a 1.5 ml tube. (See table 1 below for appropriate amounts.)
   - Cap the tube and vortex for 5 seconds. Pulse-spin the tube in a microcentrifuge to bring all liquid down from sides of the tube.
   - Using a manual single-channel pipette, add 8 µl of the Master Mix – dH_2O mixture and 2 µl dH_2O into the negative control well, i.e. the well with the negative control primer pairs, of the primer tray.
   - Using a manual single-channel pipette, add at room temperature the DNA sample to the remaining Master Mix – dH_2O mixture. (See table 1 below for appropriate amounts.)
   - Cap the tube and vortex for 5 seconds. Pulse-spin the tube in a microcentrifuge to bring all liquid down from sides of the tube.
   - Using an electronic single-channel dispenser aliquot 10 µl of the sample reaction mixture into each well, except the negative control well, of the primer tray.

Table 1: Volumes of the components needed per test for different numbers of wells when using Master Mix without *Taq* polymerase.

No. of wells per test	Volume of Master Mix (µl)	Volume of DNA sample (µl)	Volume of dH_2O (µl)	Volume of *Taq* polymerase (µl)
2	12	8	19.7	0.3
3	15	10	24.6	0.4
4	18	12	29.5	0.5
5	21	14	34.4	0.6
6	24	16	39.4	0.6
7	27	18	44.2	0.8
8	30	20	49.2	0.8
9	33	22	54.1	0.9
10	36	24	59	1.0
11	39	26	63.9	1.1
12	42	28	68.9	1.1
13	45	30	73.8	1.2
14	48	32	78.7	1.3
15	51	34	83.6	1.4
16	54	36	88.6	1.4
17	60	40	98.4	1.6
18	63	42	103.3	1.7
19	66	44	108.2	1.8
20	69	46	113.2	1.8
21	72	48	118.1	1.9
22	75	50	123	2.0
23	78	52	127.9	2.1
24	81	54	132.8	2.2

No. of wells per test	Volume of Master Mix (µl)	Volume of DNA sample (µl)	Volume of dH_2O (µl)	Volume of *Taq* polymerase (µl)
25	87	58	142.7	2.3
26	90	60	147.6	2.4
27	93	62	152.5	2.5
28	96	64	157.4	2.6
29	99	66	162.4	2.6
30	102	68	167.3	2.7
31	105	70	172.2	2.8
32	108	72	177.1	2.9
36	126	84	206.6	3.4
40	138	92	226.3	3.7
44	150	100	246	4.0
48	162	108	265.7	4.3
52	174	116	285.4	4.6
56	186	124	305	5.0
60	198	132	324.7	5.3
64	210	140	344.4	5.6
68	228	152	373.9	6.1
72	240	160	393.6	6.4
76	252	168	413.3	6.7
80	264	176	433	7.0
84	276	184	452.6	7.4
88	288	192	472.3	7.7
92	300	200	492	8.0
96	312	208	511.7	8.3

The recommended volumes listed above include volume to compensate for pipette variations and for losses of liquid on the interior walls of the tubes.

5. Combi kits A-B-DR, A-B-C, A-B-DR-DQ and DQA-DQB-DR Enhanced and the HLA-C high resolution for frequent alleles kit

- Vortex the Master Mix.
- Using a manual single-channel pipette, add at room temperature 8.3 µl *Taq* polymerase (5 units/µl) and 511.7 µl dH_2O into the provided 1.5 ml tube containing 312 µl Master Mix.
- Cap the tube and vortex for 5 seconds. Pulse-spin the tube in a microcentrifuge to bring all liquid down from sides of the tube.
- Using a manual single-channel pipette, add 8 µl of the Master Mix – *Taq* polymerase – dH_2O mixture and 2 µl dH_2O into the negative control well No. 96, i.e. the well with the negative control primer pairs.
- Using a manual single-channel pipette, add at room temperature 206 µl DNA sample to the remaining Master Mix – *Taq* polymerase – dH_2O mixture.

- Cap the tube and vortex for 5 seconds. Pulse-spin the tube in a microcentrifuge to bring all liquid down from sides of the tube.
- Using an electronic single-channel dispenser aliquot 10 µl of the sample reaction mixture into each well, except the negative control well No. 96, of the primer tray.

Important:

Be sure to apply the sample above the primers (dried at the bottom of each well of the primer tray) to avoid cross-contamination between wells. Touch the inside wall of the well with the pipette tip to allow the sample to slide down to the bottom of the well. Check that all samples have dropped to the bottom of each well. If not, tap the tray gently on the bench top so that all samples settle at the bottom of the well before you begin PCR.

5. Cover the primer tray(s) with the provided adhesive PCR seals. Check that all reaction wells are completely covered to prevent evaporative loss during PCR amplification. The *Olerup* SSP® Compression Pad (Product No. 103.505-06) can be applied on top of the adhesive PCR seals to prevent evaporation during thermal cycling.
6. Place the primer tray(s) in the thermocycler with a suitable tube-tray adapter. Do not allow more than 5 minutes delay between PCR setup and thermal cycling.
7. Enter your *Olerup* SSP® program number. Specify a 10 µl reaction volume.
8. Start the PCR program. The program takes approximately 1 hour and 20 minutes.
9. Remove the primer tray(s) from the thermocycler. Inspect the PCR tray to make sure that there is approximately the same volume of fluid in each PCR well. Electrophorese the samples, see the section E – Gel Electrophoresis below. Interpret the typing results with the ***lot-specific Interpretation and Specificity Tables or Worksheet***, see Expected Values below.

E. Gel Electrophoresis

1. After completing the PCR reaction orient the primer tray and gel box. The order of the wells is from left to right and top to bottom.
2. Gently remove the strip lids without splashing the PCR products.
3. Load the PCR products in sequence to the 2 % agarose gel. (No addition of gel loading buffer is necessary.) Use of an 8-channel pipette for gel-loading is recommended.
4. Load a DNA size marker (100 base pair ladder, DNA Size Marker Product No. 103.202-100 or DNA Size Marker for short gel runs 103.203-100) in one well per row.
5. Cover the gel box with the gel box lid
6. Electrophorese the gel in 0.5 x TBE buffer, without re-circulation of the buffer, for 15-20 minutes at 8-10 V/cm.
7. Transfer the gel tray with the gel to a UV transilluminator.
8. Photograph the gel with or without the gel tray.
9. Mark the photograph according the rules of the laboratory.

QUALITY CONTROL

ASHI HLA testing guidelines indicate that a negative (contamination) control well must be included in each PCR setup. (Standards for Accredited Laboratories, American Society for Histocompatibility and Immunogenetics, 2012 Revised Standards Approved by the ASHI Board of Directors: January 2013, Guidance Final Version October 2012). A negative control well is included in all kits from lot 01V and forward (the DQ low kit from lot 06Y and forward).

Refer to Gel Interpretation on page 14.

RESULTS

Lot-specific Cell Line Validation Sheets and Certificate of Analysis can be accessed online, https://labproducts.caredx.com/products/olerup-ssp/.

LIMITATIONS OF THE PROCEDURE

1. The PCR-SSP process requires highly controlled test conditions to ensure adequate discriminatory amplification. The procedure described in Instructions for Use must be strictly followed.
2. The extracted DNA sample is the template for the specific PCR amplification process. The purified DNA should have an $A_{260/280}$ ratio between 1.6 and 2.0 to obtain optimal band visualization by electrophoresis.
3. All instruments, e.g. thermocycler, pipetting devices, must be calibrated according to the manufacturer's recommendations.
4. Lot-specific information is given in the Product Insert: Lot-specific Information and in the lot-specific Worksheet.
5. Based on testing performed, the following substances were evaluated with three (3) extraction methods at the concentrations listed and found not to impact test performance.

Extraction Method	Interfering Substance	Interferent concentration*
EZ1 DSP DNA Blood System	Bilirubin	200mg/L
	Hemoglobin	200g/L
	Triglyceride	30g/L
	Protein	110g/L
QIAamp DSP DNA Blood Kit	Bilirubin	200mg/L
	Hemoglobin	200g/L
	Triglyceride	18.2g/L
	Protein	77 - 96g/L
Gentra PureGene method	Bilirubin	200mg/L
	Hemoglobin	200g/L
	Triglyceride	18.2g/L
	Protein	119 -146g/L

6. The PCR plates are physically compatible with the majority of thermocyclers on the market. See thermocycler plastic compatibility table below.
   Note: The table is intended as a guide only. For validated cyclers, please see section Instrument Requirements – Instrument.

Compatibility Table	
Manufacturer	**Thermocycler**
Applied Biosystems	GeneAmp 9700
	ProFlex 96-well
	ProFlex 2x96-well
	Veriti 0.2ml 96-well Block
	GeneAmp 2700, 2720, 9600
Agilent (Stratagene)	SureCycler 8800
	RoboCycler
	Gradient Cycler
Biometra	Uno, Uno II
	T1 Thermocycler
	TGradient/TAdvanced
	TRobot
	TProfessional
Bio-Rad	MJ Mini
	T100
	iCycler, MyCycler
	C1000, S1000
	PTC-2(xx)
	PTC-100 with 96-well block
Eppendorf	Mastercycler Gradient
	Mastercycler EP Gradient, Pro, Nexus
MWG	Primus 96
	TheQ Lifecycler
Thermo Scientific	Arktik
Techne	Flexigene, TC-412, TC-4000
	Genius, Touchgene, TC-512, TC-5000
	TC-PLUS, Prime, PrimeG, Prime Elite
Thermo Scientific Hybaid	PCR Express, Px2, PxE
	MultiBlock System & MBS
	Omnigene, Omn-E
Gene Technologies	GS1, GS4, GSX
Takara	TP 3000

7. The performance of this kit using the Master Mix without Taq polymerase has only been validated with the Roche Taq DNA Polymerase, GMP Grade (Catalog # 03 734 927 001 or #03 734 935 001). Assay performance using any other enzymes is unknown and must be established and validated by the user.

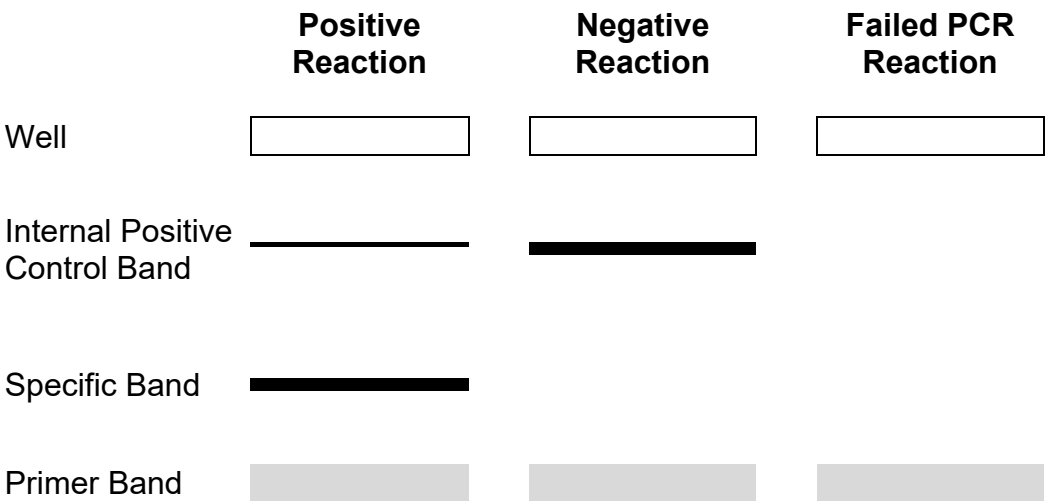
EXPECTED VALUES

A. Data Analysis

Examine the gel photo carefully and determine the positive lanes.

1. A faster-migrating, shorter band will be seen in a gel lane if specific HLA allele(s) was amplified. This indicates a positive test result.
   a. Record the presence and absence of specific PCR products.
   b. It is useful to monitor the relative lengths of the specific PCR products as given in the lot-specific product inserts when interpreting the gel results. Several lanes have two or more possible lengths of specific PCR products. These wells contain multiple primer pairs generating PCR products of different sizes depending upon the HLA allele(s) of the sample DNA.
   c. Match the pattern of gel lanes with specific PCR products with the information in the lot-specific Interpretation and Specificity Tables to obtain the HLA typing of the sample DNA.
2. An internal positive control band, slower-migrating and longer, should be visible in all gel lanes, except in the negative control gel lane, as a control of successful amplification. The internal positive control band may be weak or absent in positive gel lanes.
   a. 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.
   b. Absence of internal positive control band with no specific PCR product indicates failed PCR reaction.
      i. If HLA alleles can be determined in the presence of failed PCR reaction(s) and the failed PCR reaction(s) does not change the allele assignment, then the test does not have to be repeated.
      ii. If, however, if the failed PCR reaction(s) could change the HLA allele assignment, then the typing must be repeated.
3. The presence of specific PCR product or internal positive control band in negative control lane(s) indicates contamination with PCR product(s) and voids all test results. Primer oligomers ranging from 40 to 60 base pairs in size might be observed in the negative control lane(s). This does not represent contamination.

B. Gel Interpretation



1. A DNA size marker (100 base pair ladder, DNA Size Marker Product No. 103.202-100 or DNA Size Marker for short gel runs 103.203-100) should be run in one well per row of the gel or according to local laboratory accreditation guidelines.
2. Bands longer than the internal positive control band might be obtained and these should be disregarded in the interpretation of the typing results.
3. Unused primers will form a diffuse band shorter band 50 base pairs.
4. Primer oligomer artefacts might be observed. These are longer than the primer band but shorter than the specific bands.

SPECIFIC PERFORMANCE CHARACTERISTICS

Kit Lot Quality Control

Each primer solution is tested against a panel of 48 DNA samples from well characterized cell lines of the IHWC, see the lot-specific Cell Line Validation Sheet(s) in the Product Insert, Lot-specific Information.

Method Comparison

A study was conducted to compare the *Olerup* SSP® HLA Typing Kits with and without *Taq* polymerase provided in the PCR Master Mix. The purpose of the study was to demonstrate that manual addition does not affect assay results and that different *Taq* polymerase lots yield the same results. The study included HLA-A, -B (Class I) and -DRB (Class II) low resolution typing of 15 DNA samples with known HLA genotype obtained from the International Histocompatibility Workshop Collection. Samples were prepared and coded by personnel different from those performing the testing; the testing personnel were blinded to the samples' HLA types. Each sample was tested in parallel

with the *Olerup* SSP® HLA-A-B-DR Combi Tray with PCR Master Mix without *Taq* polymerase ("Test") and the *Olerup* SSP® HLA-A-B-DR Combi Tray with PCR Master Mix including *Taq* polymerase ("Control"), in accordance with each assay's product insert instructions. All testing was performed at *Olerup* SPP AB Laboratory in Stockholm, Sweden, using one kit lot and two *Taq* polymerase lots. The Roche *Taq* DNA polymerase, GMP Grade, was used in the study. Testing on each sample consisted of low resolution typing with the Olerup SSP® HLA-A-B-DR Combi Tray performed with: 1) PCR Master Mix including Taq polymerase (FDA-cleared assay) (Control assay), 2) PCR Master Mix plus Roche Taq DNA polymerase, GMP Grade product, 5 U/µL; Lot #1 (Test Lot #1), and 3) PCR Master Mix plus Roche Taq DNA polymerase, GMP Grade product, 5 U/µL; Lot #2 (Test Lot #2). After PCR, detection was performed using gel electrophoresis. The results are summarized below.

Comparison	Agreement			
	Class I Typing Results (# agreement/total)		Class II Typing Results (# agreement/total)	% Agreement (95% Confidence Interval[a])
	HLA-A	HLAB	HLA-DRB	
Test Lot #1 / Control Lot	15/15	15/15	15/15	100% (84.7 – 100.0%)
Test Lot #1 / Consensus[b]	15/15	15/15	15/15	100% (84.7 – 100.0%)
Test Lot #2 / Control Lot	15/15	15/15	15/15	100% (84.7 – 100.0%)
Test Lot #2 / Consensus	15/15	15/15	15/15	100% (84.7 – 100.0%)
Test Lot #1 / Test Lot #2	15/15	15/15	15/15	100% (84.7 – 100.0%)

[a] Score Method
[b] Consensus result from the International Histocompatibility Workshop Collection

BIBLIOGRAPHY

1. Olerup O, Zetterquist H. *HLA-DRB1*01* subtyping by allele-specific PCR-amplification: A sensitive, specific and rapid technique. *Tissue Antigens* 1991: **37**: 197-204.
2. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantations. *Tissue Antigens* 1992: **39**: 225-235.
3. Current HLA alleles can be found at:
   a. http://www.ebi.ac.uk/imgt/hla or
   b. http://www.anthonynolan.org.uk/HIG.

TROUBLESHOOTING

Problem	Reason	Action
No amplification (neither amplification of the internal control fragments, nor specific amplifications).	Too low amount of DNA.	Measure the DNA concentration and see if the amount added is correct. RNA contamination may cause a spectrophotometric overestimation of DNA concentration. Repeat the DNA extraction carefully with freshly prepared solutions. We recommend automated DNA extraction with the QIAGEN EZ1 DSP DNA Blood System.
	The DNA contains PCR inhibitors, e.g. proteins, ethanol (from precipitation steps), remaining matrixes from solid-phase DNA purification products.	Measure the DNA quality. We recommend an A260/A280 ratio of 1.6 – 2.0 by UV spectrophotometry. Follow the supplier's DNA extraction protocol exactly. Re-extract the DNA. We recommend automated DNA extraction with the QIAGEN EZ1 DSP DNA Blood System.
	The DNA has been extracted from heparinized blood.	Use non-heparinized blood or use DNA extraction protocols for heparinized blood.
	The DNA is dissolved in a buffer containing EDTA.	Repeat the DNA extraction and dissolve the DNA in dH_2O.

Problem	Reason	Action
Continuing: **No amplification (neither amplification of the internal control fragments, nor specific amplifications).**	Accidental introduction of bleach into test.	Review areas where bleach might possibly be introduced.
	Kits are not stored at adequate temperature.	Store the kits at -20°C.
	Thermal cycler is not working in a proper way.	Calibrate the thermal cycler and check the PCR program. A thermal cycler used for routine PCR-SSP typing should be calibrated every 6-12 months.
	Inadequate contact between thermal cycler heating block and SSP typing tray.	Use correct tray/retainer for 0.2 ml thin-walled reaction wells, refer to the thermal cycler manual.
Random failure of amplification (drop-outs).	PCR seals/PCR tube caps that are not tightly closed will lead to evaporation and subsequent failure of amplification.	Make sure the PCR seals/all caps are tightly closed. The *Olerup* SSP® Compression Pad (Product No. 103.505-06) can be applied on top of the adhesive PCR seals to prevent evaporation during thermal cycling.
	Gel-loading mistakes.	Check that the correct number of wells has been loaded and that each well contains approximately the same volume of PCR mixture.
	Use of non-calibrated pipettes.	Calibrate all pipettes routinely according to the supplier's recommendations.
	Pipetting errors.	Perform pipetting more carefully.
	Master Mix and sample DNA have not been properly mixed before use.	Mix briefly by vortexing before use. We recommend to vortex after each row.
	Uneven volume of DNA-Master Mix mixture has been added to the wells.	Perform pipetting more carefully.

Problem	Reason	Action
Weak internal control fragments.	Impure DNA.	Measure the DNA quality. The A260/A280 ratio should be 1.6 – 2.0 by UV spectrophotometry. RNA contamination may cause a spectrophotometric overestimation of DNA concentration. Degraded DNA give rise to smear in gel lanes. Repeat the DNA extraction carefully with freshly prepared solutions. We recommend automated DNA extraction with the QIAGEN EZ1 DSP DNA Blood System.
	Too low amount of DNA.	Measure the DNA concentration and adjust to 30 ng/µl or to 15 ng/µl for DNA extracted by the QIAGEN EZ1 DSP DNA Blood System. RNA contamination may cause a spectrophotometric overestimation of DNA concentration. Degraded DNA give rise to smear in gel lanes. Repeat the DNA extraction carefully with freshly prepared solutions. We recommend automated DNA extraction with QIAGEN EZ1 DSP DNA Blood System.

Problem	Reason	Action
Continuing: **Weak internal control fragments.**	Too high annealing temperature, the thermal cycler is not calibrated.	Calibrate the thermal cycler and check the PCR program. A thermal cycler used for routine PCR-SSP typing should be calibrated every 6-12 months.
	The PCR Master Mix has been stored at +4°C for longer than 2 weeks.	Properly store PCR Master Mix.
Non-specific amplification (ladders or smears).	Use of excess DNA sample.	Measure the DNA concentration and adjust 30 ng/µl or to15 ng/µl for DNA extracted by the QIAGEN EZ1 DSP DNA Blood System. Some primer solutions have a higher tendency of giving rise to non-specific amplification, see footnotes in each lot-specific Specificity Table.
	Impure DNA.	All fragments larger than the internal control fragment should be disregarded when interpreting the obtained results. Check the DNA quality. Repeat the DNA extraction. We recommend automated DNA extraction with the QIAGEN EZ1 DSP DNA Blood System. Some primer solutions have a higher tendency of giving rise to non-specific amplification, see footnotes in each lot-specific Specificity Table.

Problem	Reason	Action
Weaker and weaker amplification signals over time.	The ethidium bromide agarose gel staining solution is old.	Prepare fresh ethidium bromide solution to achieve better staining of the agarose gel and better signal. The primer clouds are easy to detect if the staining of the agarose gel is normal.
	One of the UV lamps is broken.	Check the UV light equipment. The primer clouds are easy to detect if the UV light is normal.
	Used too little DNA sample.	Measure the DNA concentration and adjust to 30 ng/µl or to 15 ng/µl for DNA extracted by the QIAGEN EZ1 DSP DNA Blood System.
	Too high annealing temperature, the thermal cycler is not calibrated.	Calibrate the thermal cycler and check the PCR program. A thermal cycler used for routine PCR-SSP typing should be calibrated every 6-12 months.
Strange amplification patterns.	Incorrect lot-specific Interpretation Table / worksheet is used.	Check the lot number of the product used and the Interpretation Table / worksheet used.
	Incorrect order in gel loading.	Check alignment of mixes and gel lanes.
	The amplification pattern contains a false positive.	See below.
	The amplification pattern contains a false negative.	See below.

Problem	Reason	Action
False positive amplifications.	DNA contamination.	Use gloves, pipette tips containing barriers (filter plugs) and separate rooms for pre-PCR handling and post-PCR handling. Assure accurate handling of all samples, in all steps. Check for contamination using *Olerup* SSP® Wipe Test kit.
	Impure DNA.	Measure DNA quality. Follow the supplier's DNA extraction protocol exactly. Try other DNA extraction systems. Re-extract the DNA. We recommend automated DNA extraction with the QIAGEN EZ1 DSP DNA Blood System.
	Use of excess DNA sample.	Measure the DNA concentration and adjust to 30 ng/µl or to 15 ng/µl for DNA extracted by the QIAGEN EZ1 DSP DNA Blood System.
	Too low annealing temperature.	Calibrate the thermal cycler and check the PCR program. A thermal cycler used for routine PCR-SSP typing should be calibrated every 6 -12 months.
	Extensive delay between PCR setup and start of thermal cycling.	No more than a 5-minute delay should be allowed before thermal cycling.
	Delay between placing typing trays in thermal cycler and start of cycling.	Use pre-heated thermal cycler.

Problem	Reason	Action
Continuing: **False positive amplifications.**	Use of excess ethidium bromide.	Use recommended amount of ethidium bromide.
	Incorrect interpretation of an artefact as a specific band.	Check the lot-specific Interpretation Table / worksheet and Specificity Table for correct band size and foot notes.
	The amplification pattern contains a false positive.	Check if all specific amplifications are correct in size or if an artefact (carry-over, primer dimer) has been misinterpreted as an amplification.
	Incorrect order in gel loading.	Check alignment of mixes and gel lanes.
False negative amplifications.	The thermal cycler is not properly calibrated.	Calibrate the thermal cycler and check the PCR program. A thermal cycler used for routine PCR-SSP typing should be calibrated every 6-12 months. If not corrected by re-calibration, re-type the test with a reference sample of the same specificity. If confirmed negative, contact customer support.
	Incorrect order in gel loading.	Check alignment of mixes and gel lanes.

Problem	Reason	Action
Overall gel problems (fuzzy gels and/or smeared lanes).	Degraded DNA sample.	Appears as a smear in the gel lanes. Isolate DNA from a fresh sample.
	Heavy streaking in random wells.	Uneven suspensions of DNA. Make sure sample DNA is dissolved before taking your aliquot. Vortex diluted DNA sample.
	PCR product floated out of well.	Carefully align pipette tips with gel wells and dispense slowly.
	The electrophoresis buffer might be too warm.	Prepare new TBE buffer. Run at a lower voltage.
	Incorrect percentage agarose gel has been used.	Make sure the recommended 2% agarose gel is used.
	Agarose not completely dissolved.	Shortly re-boil to melt the agarose.
	Incorrect TBE concentration.	Use the recommended 0.5 x TBE concentration.
	Gels too newly casted.	Gels are not ready for use until 15 minutes after casting.
	Gels too old.	Do not cast gels too far in advance.
	The gel comb used has too thick slots.	Use thin combs (4 x 1 mm).
	Gel tray not UV transparent.	Remove gel from gel tray before viewing.
	Gel picture too bright.	Excess use of ethidium bromide. Check the camera settings.
	Gel picture too dark.	Use recommended amount of ethidium bromide. Check the camera settings.

Problem	Reason	Action
General problems with false negative amplification or run-to-run dependent problems of such nature	Ramp rate setting too high.	Olerup SSP kits are validated using GeneAmp 9700 cycler set to the 9600 mode and ProFlex with a ramp rate of 3°C/s. Higher ramp rates than the equivalent to that may have an effect on the typing results.

TRADEMARKS USED IN THIS DOCUMENT/PRODUCT

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

NOTICE TO PURCHASER: The *Olerup* SSP® Kits without *Taq* Polymerase – This product is optimized for use with the Roche *Taq* Polymerase, GMP Grade (Catalog # 03 734 927 001 or #03 734 935 001) in the Polymerase Chain Reaction ("PCR") Process which may be covered by patents by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd. ("Roche"). The laboratory is responsible for contacting Roche Molecular Systems, Inc. to determine if a license is needed under these patents.

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*, Franzéngatan 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 worksheet, lot-specific Specificity and Interpretation Tables of the product insert.

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Changes in revision 0193-LBL v04 compared to 0193-LBL v03:
1. Contact information revised.

Changes in revision 0193-LBL v03 compared to 0193-LBL v02:
1. Date of revision added.
2. Information regarding the lot-specific product insert documents clarified.
3. CareDx Pty Ltd added as distributor.