Olerup SSP™ HLA Wipe Test – Negative Control

Product number:	**102.101-01 – licensed for PCR** **102.101-01u – not licensed for PCR**
Lot number:	**X24**
Expiry date:	**2008-October-01**
Number of tests:	**96**
Number of tubes per test:	**1-2**
Storage - pre-aliquoted primers:	**dark at -20°C**
- Positive Control DNA:	**-20°C**
- PCR Master Mix:	**-20°C**

This Product Description is only valid for Lot No. X24.

GENERAL DESCRIPTION

The *Olerup* SSP™ HLA Wipe Test – Negative Control is intended to be used to monitor for contamination with amplicons generated by the *Olerup* SSP™ product line and can also be used as a negative control in *Olerup* SSP™ typings.

The primer set contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup* SSP™ HLA Class I, DRB, DQB1 and DPB1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

The *Olerup* SSP™ HLA Wipe Test – Negative Control has the sensitivity to detect approximately 50 copies of DNA template.

PRODUCT DESCRIPTION

HLA Wipe Test – Negative Control

CONTENT

The primer set contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup* SSP™ HLA Class I, DRB, DQB1 and DPB1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

PCR product sizes range from 75 to 430 base pairs.

Length of PCR product	**105**	**200**	**105**	**80**	**75**	**80**
5'-primer[1]	**164**	**340**	**440**	**45**	**45**	**43**
	5'-CAC3'	**5'-Agg3'**	**5'-TTA3'**	**5'-Tg g3'**	**5'-Tg g3'**	**5'-Tg g3'**
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**
A*	**+**	**+**	**+**			
B*	**+**	**+**	**+**			
Cw*	**+**	**+**	**+**			
DRB1				**+**	**+**	
DRB3				**+**	**+**	
DRB5				**+**		
DQB1					**+**	
DPB1						**+**

[1]The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as in *Tissue Antigens* 1998, 51:II, 417-466. The sequence of the 3 terminal nucleotides of the primer is given.

[2]The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as in *Tissue Antigens* 1998, 51:II, 417-466. The sequence of the 3 terminal nucleotides of the primer is given.

The primer solution is pre-aliquoted into 0.2 ml PCR tubes. Each tube contains the same dried primer solution.
PCR Master Mix complete with Taq, *Taq* polymerase, nucleotides, buffer, glycerol and cresol red, as well as PCR lids are included in the licensed kit.
PCR Master Mix without Taq, nucleotides, buffer, glycerol and cresol red, as well as PCR lids are included in the unlicensed kit.
1-2 PCR reactions with a reaction volume of 10 µl are performed per test.
Positive Control DNA is included in the kit, 20 ng/µl, 75 µl.
Sterile swabs, Dacron fiber tipped, plastic applicator are included in the kit. One per envelope. 100 per kit.
Note: The pellets in the tubes may vary in form and colour. This does not affect the performance of the product.

PLATE LAYOUT

Each test consists of 1-2 PCR reactions. Each well of the 8 well PCR plates contains the same primer mix.

1	**1**	**1**	**1**	**1**	**1**	**1**	**1**

The 8 well cut PCR plate is marked with 'Wipe Test'.
Well No. 1 is marked with the Lot No. 'X24'.
The PCR plates are covered with a PCR-compatible aluminum foil.

Please note: When removing each PCR well, make sure that the remaining plates/wells stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates/wells.

LICENSES

102.101-01 – licensed for PCR.
Notice to purchaser: Limited License.

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

102.101-01u – not licensed for PCR.
Notice to purchaser: Disclaimer of License.

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

102.101-10 and 102.101-01u

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

GUARANTEE

Olerup SSP AB guarantees that the primers in the HLA Wipe Test – Negative Control kit have the specificities stated in the product description.
When stored at –20°C, the dried primers are stable for 22 months from the date of manufacture.
When stored at –20°C, the PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* are stable for 24 months from the date of manufacture.
The kit is shipped at ambient temperature.

PROTOCOL

It is recommended that 10 to 12 commonly used areas are tested for contamination; DNA preparation area, PCR setup area and post-amplification area. Test e.g. work benches, pipettes, centrifuges, refrigerator and freezer handles, door knobs, racks.

1. In a DNA free location label one 1.5 ml tube for each of the sample areas.
2. Add 500 µl sterile, distilled water to each tube.
3. Wet one of the provided sterile plastic applicator swab in each tube.
4. Wipe the area to be tested with the moistened applicator, and place it back in the original tube. Snap off the plastic stem of the applicator and close the cap of the tube.
5. Vortex briefly.
6. Incubate the samples at 55^{o}C in a waterbath or heating block for 1 hour.
7. Centrifuge, 1 minute, 10 000 to 13 000 rpm in a microcentrifuge.
8. Remove and discard the applicator from the tubes with sterile forceps.

PCR AMPLIFICATION

Positive control well.
Add 1 µl of the provided Positive Control DNA and 1 µl of dH_2O to well 1.

Negative control well.
Add 2 µl of dH_2O to well 2.

For each test area run two wells.
Add to the first well for each test area; 1 µl of the test sample and 1 µl of dH_2O.
Add to the second well for each test area; 1 µl of the test sample and 1 µl of the Positive Control DNA. This well serves as an inhibition control.

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In a 0.5 ml tube mix:

3 µl of PCR Master Mix complete with *Taq* x the number of wells + 2
and
5 µl of dH_2O x the number of wells + 2

e.g. for a Wipe Test consisting of (i) positive control well, (ii) negative control well and (iii) 10 tested areas (2 x 10 wells) mix:
24 x 3 = 72 µl of PCR Master Mix complete with *Taq* and
24 x 5 = 120 µl of dH_2O

Mix well, dispense 8 µl of the PCR Master Mix complete with *Taq*-H_2O mixture into each of the wells of the HLA Wipe test. ***Well No. 1 of the 8 well PCR plates is marked with the lot number.*** Close the 8 well PCR plate(s) with the provided lids.

The HLA Wipe Test – Negative Control can also be used as a negative control when using the *Olerup* SSP™ kits.

Cut off one well with a pair of scissors. Add to this well;
3 µl of PCR Master Mix complete with *Taq*
7 µl of dH_2O

Include this well as a negative control when performing *Olerup* SSP™ typings.

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In a 0.5 ml tube mix:

3 μl of PCR Master Mix without *Taq* x the number of wells + 2,
0.1 μl of Taq polymerase (5 units/μl) x the number of wells + 2 and
5-0.1 μl of dH_2O x the number of wells + 2

e.g. for a Wipe Test consisting of (i) positive control well, (ii) negative control well and (iii) 10 tested areas (2 x 10 wells) mix:
24 x 3 = 72 μl of PCR Master Mix without *Taq* and
24 x 0.1 = 2.4 μl of *Taq* polymerase
24 x 5 – 2.4 = 117.6 μl of dH_2O

Mix well, dispense 8 μl of the PCR Master Mix without *Taq*-*Taq*-H_2O mixture into each of the wells of the HLA Wipe test. ***Well No. 1 of the 8 well PCR plates is marked with the lot number.*** Close the 8 well PCR plate(s) with the provided lids.

The HLA Wipe Test – Negative Control can also be used as a negative control when using the *Olerup* SSP™ kits.

Cut off one well with a pair of scissors. Add to this well;
3 μl of PCR Master Mix without *Taq*
0.1 μl of Taq polymerase (5 units/μl)
6.9 μl of dH_2O

Include this well as a negative control when performing *Olerup* SSP™ typings.

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

PCR cycling parameters:

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

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

AGAROSE GEL ELECTROPHORESIS

Prepare a 2% (w/v) agarose gel in 0.5 x TBE buffer. Dissolve the agarose by boiling in a microwave oven. Let the gel solution cool to 60°C. Stain the gel prior to casting with ethidium bromide (10 mg/ml), 5 µl per 100 ml gel solution. For maximal ease of handling use our ethidium bromide dropper bottles (Product No. 103.301-10), 1 drop of ethidium bromide solution per 50-75 ml of gel. **<u>Note:</u> Ethidium bromide is a powerful carcinogen.**

Load the PCR products, preferably using an 8-channel pipette. Load a DNA size marker (100 base pair ladder, Product No. 103.201-100) in one well per row.

Run the gel in 0.5 x TBE buffer, without re-circulation of the buffer, for 15-20 minutes at 8-10 V/cm.

DOCUMENTATION AND INTERPRETATION

Put the gel on a UV transilluminator and document by photography.
Record the presence and absence of PCR products.

HLA Wipe Test

In the positive control lane one or several PCR products should be seen.

In the negative control lane no PCR product should be seen[1].

The presence of PCR product(s) in the sample lanes without Positive Control DNA indicates contamination[1].
The absence of PCR product in the sample lanes without Positive Control DNA shows that no detectable contamination is present

In the sample lanes with the Positive Control DNA PCR product(s) of equal strength as in the positive control lane should be seen. If these PCR product(s) are weaker than the PCR product(s) in the positive control lane, then an inhibitor may be present in the sample. The test should then be repeated with the sample diluted 1:50 in sterile dH2O.

If contamination is detected, clean the area with a fresh 10% bleach solution and re-test the area.

Negative Control

In the negative control well no PCR product should be seen. The presence of PCR product(s) indicates contamination[1].

If contamination is detected, wipe test and testing of all reagents should be performed in order to detect the source of contamination.

[1]Primer oligomer artifacts may occasionally be seen. This does not represent contamination.

PCR MASTER MIXES

The PCR Master Mix complete with *Taq* contains:

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

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

The PCR Master Mix without *Taq* contains:

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

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

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

CERTIFICATE OF ANALYSIS

Olerup SSP™ HLA Wipe Test – Negative Control

Product number:	**102.101-01 – licensed for PCR** **102.101-01u – not licensed for PCR**
Lot number:	**X24**
Expiry date:	**2008-October-01**
Number of tests:	**96**
Number of tubes per test:	**1-2**

Tube specification:

Tube No.	Production No.
1	2006-148-01

The negative control primer solution has been tested in a dilution series of the corresponding PCR products, 1 to 10^3 down to 1 to 10^9.

Results: The negative control primer pairs can detect contamination with the corresponding PCR products diluted 1 to 10^7.

Date of approval: 2006-December-15

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP™ HLA Wipe Test – Negative Control
Product number: 102.101-01, 102.101-01u
Lot number: X24

Intended use: Detection of contamination with HLA amplicons.

Manufacturer: *Olerup* SSP AB
Hasselstigen 1
SE-133 33 Saltsjöbaden, Sweden
Phone: +46-8-717 88 27
Fax: +46-8-717 88 18

We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2000 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex III, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

Saltsjöbaden, Sweden
2006-December-15

Olle Olerup
Managing Director

WARRANTY

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

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

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

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

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

ADDRESSES:

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

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

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

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