Olerup SSP® HLA - Negative Control SSP

Product number:	**102.102-01u – without *Taq* polymerase**
Lot number:	**81M**
Expiry date:	**2014-April-01**
Number of tests:	**96**
Number of wells per test:	**1**
Storage - pre-aliquoted primers:	**dark at -20°C**
- PCR Master Mix:	**-20°C**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 81M.

GENERAL DESCRIPTION

The *Olerup* SSP® HLA– Negative Control is intended to 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 – Negative Control has the sensitivity to detect approximately 50 copies of DNA template.

PRODUCT DESCRIPTION

HLA - Negative Control SSP

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.
The PCR product generated by the control primer pair is 430 base pairs.

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

[1]The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and 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.

The primer solution is pre-aliquoted into 0.2 ml PCR wells. Each well contains the same dried primer solution.
PCR Master Mix without Taq, nucleotides, buffer, glycerol and cresol red, as well as PCR lids are included in the kit without *Taq* polymerase.
1 PCR reaction with a reaction volume of 10 µl is performed per test.

PLATE LAYOUT

Each test consists of 1 PCR reaction. 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 'NC' in silver/gray ink.
Well No. 1 is marked with the Lot No. '81M'.
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 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.

PROTOCOL

PCR AMPLIFICATION

For users of Olerup SSP® kits without Taq polymerase

Cut off one well from the 8 well PCR plate.
Add 2 µl dH_2O to the negative control well.
Add 8 µl of the PCR Master Mix-*Taq*-H_2O mixture to the negative control well, i.e. before the sample DNA is added to the PCR Master Mix-*Taq*-H_2O mixture.

Add the sample DNA to the PCR Master Mix-*Taq*-H_2O mixture, mix well and dispense 10 µl of the DNA-PCR Master Mix-*Taq*-H_2O mixture into each of the wells of the SSP typing, but not into the negative control well.

The same PCR Master Mix without *Taq*, *Taq* polymerase and dH_2O that is used for the typings should be used in the negative control well. (The PCR Master Mix without *Taq* supplied with the Negative Control kit is intended to replace the PCR Master Mix used from the typing kits without *Taq p*olymerase.)

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

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

AGAROSE GEL ELECTROPHORESIS

Prepare a 2% (w/v) agarose gel in 0.5 x TBE buffer. Dissolve the agarose by boiling in a microwave oven. Let the gel solution cool to 60°C. Stain the gel prior to casting with ethidium bromide (10 mg/ml), 5 µl per 100 ml gel solution. For maximal ease of handling use our ethidium bromide dropper bottles (Product No. 103.301-10), 1 drop of ethidium bromide solution per 50-75 ml of gel. **Note: Ethidium bromide is a powerful carcinogen.**
Load the PCR products, preferably using an 8-channel pipette. Load a DNA size marker (100 base pair ladder, Product No. 103.201-100) in one well per row.
Run the gel in 0.5 x TBE buffer, without re-circulation of the buffer, for 15-20 minutes at 8-10 V/cm.

DOCUMENTATION AND INTERPRETATION

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

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 be seen. This does not represent contamination.

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA - Negative Control SSP

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Lot number: **81M**
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Number of wells per test: **1**

Well specification:

Well No.	Production No.
1	2011-928-01

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: 2011-November-09

Approved by:

Production Quality Control

Declaration of Conformity

Product name: *Olerup* SSP® HLA - Negative Control
Product number: 102.102-01u
Lot number: 81M

Intended use: Negative Control in *Olerup* SSP® HLA typings.

Manufacturer: *Olerup* SSP AB
Franzengatan 5
SE-112 51 Stockholm, Sweden
Phone: +46-8-717 88 27
Fax: +46-8-717 88 18

We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2008 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex III, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at *Olerup* SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.

Stockholm, Sweden
2011-November-09

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

ADDRESSES:

Manufacturer:
***Olerup* SSP AB**, Franzengatan 5, SE-112 51 Stockholm, Sweden.
Tel: +46-8-717 88 27
Fax: +46-8-717 88 18
E-mail: info-ssp@olerup.com
Web page: http://www.olerup-ssp.com

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

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

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