

## **Olerup SSP<sup>®</sup> HLA - Negative Control SSP**

Product number:	102.102-01 – including <i>Taq</i> 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<sup>®</sup>* HLA– Negative Control is intended to be used as a negative control in *Olerup SSP<sup>®</sup>* typings.

The primer set contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup SSP<sup>®</sup>* 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<sup>®</sup>* 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<sup>®</sup> 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
<b>5'-primer<sup>1</sup></b>	<b>164</b>	<b>340</b>	<b>440</b>	<b>45</b>	<b>45</b>	<b>43</b>
	5'-CAC <sup>3'</sup>	5'-Agg <sup>3'</sup>	5'-TTA <sup>3'</sup>	5'-Tg g <sup>3'</sup>	5'-Tg g <sup>3'</sup>	5'-Tg g <sup>3'</sup>
<b>3'-primer<sup>2</sup></b>	<b>231</b>	<b>2<sup>nd</sup> I</b>	<b>507</b>	<b>59</b>	<b>58</b>	<b>57</b>
	5'-TgC <sup>3'</sup>	5'-AAA <sup>3'</sup>	5'-TTg <sup>3'</sup>	5'-CTC <sup>3'</sup>	5'-ggC <sup>3'</sup>	5'-CTC <sup>3'</sup>
<b>A*</b>	<b>+</b>	<b>+</b>	<b>+</b>			
<b>B*</b>	<b>+</b>	<b>+</b>	<b>+</b>			
<b>C*</b>	<b>+</b>	<b>+</b>	<b>+</b>			
<b>DRB1</b>				<b>+</b>	<b>+</b>	
<b>DRB3</b>				<b>+</b>	<b>+</b>	
<b>DRB5</b>				<b>+</b>		
<b>DQB1</b>					<b>+</b>	
<b>DPB1</b>						<b>+</b>

<sup>1</sup>The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2<sup>nd</sup> or 3<sup>rd</sup> 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](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>2</sup>The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2<sup>nd</sup> or 3<sup>rd</sup> exon or the 2<sup>nd</sup> 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](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

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Lot-specific Information

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The primer solution is pre-aliquoted into 0.2 ml PCR wells. Each well 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 kit including *Taq* polymerase.

### 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
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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 including Taq polymerase***

Cut off one well from the 8 well PCR plate.

Add 2 µl dH<sub>2</sub>O to the negative control well.

Add 8 µl of the PCR Master Mix complete with *Taq*-H<sub>2</sub>O mixture to the negative control well, i.e. before the sample DNA is added to the PCR Master Mix complete with *Taq*-H<sub>2</sub>O mixture.

Add the sample DNA to the PCR Master Mix complete with *Taq* -H<sub>2</sub>O mixture, mix well and dispense 10 µl of the DNA-PCR Master Mix complete with *Taq*-H<sub>2</sub>O mixture into each of the wells of the SSP typing, but not into the negative control well.

The same PCR Master Mix Complete with *Taq* and the same dH<sub>2</sub>O that is used for the typings should be used in the negative control well. (The PCR Master Mix complete with *Taq* supplied with the Negative Control kit is intended to replace the PCR Master Mix used from the typing kits including *Taq* polymerase.)

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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<sup>1</sup>.

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

<sup>1</sup>Primer oligomer artifacts may be seen. This does not represent contamination.

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## CERTIFICATE OF ANALYSIS

### **Olerup SSP<sup>®</sup> HLA - Negative Control SSP**

Product number: 102.102-01 – including *Taq* polymerase  
Lot number: 81M  
Expiry date: 2014-April-01  
Number of tests: 96  
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<sup>3</sup> down to 1 to 10<sup>9</sup>.

**Results:** The negative control primer pairs can detect contamination with the corresponding PCR products diluted 1 to 10<sup>7</sup>.

**Date of approval:** 2011-November-09

**Approved by:**

**Production Quality Control**

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Lot-specific Information

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## Declaration of Conformity

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

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

**Manufacturer:** *Olerup* SSP AB  
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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

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Lot-specific Information

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