Lot No.: 3K2 Lot-specific information

Olerup SSP® HLA - Negative Control SSP

Product number:	**102.102-01 – including *Taq* polymerase**
	102.102-01u – without *Taq* polymerase
Lot number:	**3K2**
Expiry date:	**2023-10-01**
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. 3K2.

The HLA-Wipe test and the HLA-Negative Control contain the same primer mix.

The 8 well cut PCR plate is marked with 'WT' in silver/gray ink.

The inner box is labelled with HLA - Wipetest – Negative Control SSP.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® NEGATIVE CONTROL SSP (5H5)

One 3'-control primer was removed.

GENERAL DESCRIPTION

The *Olerup* SSP® HLA – Negative Control is intended to be used as a negative control in *Olerup* SSP® typing kits.

As of lot series V, the Negative Control is included in most *Olerup* SSP® typing kits.

The primer set contains Negative Control primer pairs, that will amplify a 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.

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

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PRODUCT DESCRIPTION

HLA - Negative Control SSP

CONTENT

The primer set contains Negative Control primer pairs, that will amplify a 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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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 adhesive PCR seals are included in the kit including *Taq* polymerase.

PCR Master Mix without Taq, nucleotides, buffer, glycerol and cresol red, as well as adhesive PCR seals 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 ‘WT’ in silver/gray ink.
Well No. 1 is marked with the Lot No. ‘3K2’.
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.

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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_2O to the negative control well.
Add 8 µl of the PCR Master Mix complete with *Taq*-H_2O mixture to the negative control well, i.e. before the sample DNA is added to the PCR Master Mix complete with *Taq*-H_2O mixture.

Add the sample DNA to the PCR Master Mix complete with *Taq*-H_2O mixture, mix well and dispense 10 µl of the DNA-PCR Master Mix complete with *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 Complete with *Taq* and the same dH_2O 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.)

As of lot series V, the Negative Control is included in most *Olerup* SSP® typings.

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* polymerase.)

As of lot series V, the Negative Control is included in most *Olerup* SSP® typings.

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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, 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, DNA Size Marker 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 PCR products.

In the negative control well no PCR product should be seen. The presence of PCR product(s) 75 to 430 bp in size indicates contamination. Primer oligomer artefacts, approximately 40 to 50 bp in size, may be seen. This does not represent contamination.

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

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