102.101-01 – including *Taq* polymerase, IFU-01
102.101-01u - without Tag polymerase, IFU-02

Visit www.olerup-ssp.com for **"Instructions for Use" (IFU)**

Lot No.: 36V **Lot-specific information** **www.olerup-ssp.com**

Olerup SSP® HLA Wipe Test – Negative Control

Product number:	**102.101-01 – including *Taq* polymerase**
Product number:	**102.101-01u – without *Taq* polymerase**
Lot number:	**36V**
Expiry date:	**2016-August-01**
Number of tests:	**96**
Number of wells per test:	**1-2**
Storage - pre-aliquoted primers:	**dark at -20°C**
- Positive Control DNA:	**-20°C**
- PCR Master Mix:	**-20°C**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 36V.

Primer pairs for the DQA1 alleles have been included in the Negative Control.

The format of the Product Insert and Worksheet have been changed.

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, 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 Wipe Test – Negative Control has the sensitivity to detect approximately 50 copies of DNA template.

102.101-01 – including *Taq* polymerase, IFU-01
102.101-01u - without Tag polymerase, IFU-02

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"Instructions for Use" (IFU)

Lot No.: 36V **Lot-specific information** **www.olerup-ssp.com**

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, 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 430 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 codonnumbering 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 complete with Taq, *Taq* polymerase, nucleotides, buffer, glycerol and cresol red, as well as adhesive PCR seals are included in the 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.

PCR Master Mix without Taq, nucleotides, buffer, glycerol and cresol red, as well as adhesive PCR seals are included in the 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.

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 'WT' in silver/gray ink.
Well No. 1 is marked with the Lot No. '36V'.
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

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 and 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.

102.101-01 – including Taq polymerase

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 seals.

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.

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

102.101-01u – without Taq polymerase

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 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 seals.

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.

As of lot series V, the Negative Control is included in the *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, 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.**
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.

HLA Wipe Test

In the positive control lane one or several PCR products should be seen. The sizes may range from 75 to 430 bp.

In the negative control lane no PCR product should be seen. Primer oligomer artifacts, approximately 40 to 50 bp in size, may be seen. This does not represent contamination.

The presence of PCR product(s) 75 to 430 bp in size in the sample lanes without Positive Control DNA indicates contamination.
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. Primer oligomer artifacts, 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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