Olerup SSP™ DRB1*10

Product number:	**101.129-06 – licensed for PCR** **101.129-06u – not licensed for PCR**
Lot number:	**Y61**
Expiry date:	**2010-February-01**
Number of tests:	**6**
Number of tubes per test:	**3**
Storage - pre-aliquoted primers:	**dark at -20°C**
- PCR Master Mix:	**-20°C**

This Product Description is only valid for Lot No. Y61.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP™ DRB1*10 LOT

The DRB1*10 primer set, specificity and interpretation tables are unchanged compared to the previous *Olerup* SSP™ DRB1*10 lot **(Lot No. Y32)**.

PRODUCT DESCRIPTION

DRB1*10 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*1001 to DRB1*1002 alleles.
The primer solutions are pre-aliquoted into 0.2 ml PCR tubes. Each tube in the set contains a dried primer solution consisting of a specific primer mix, i.e. allele- and group-specific primers as well as a ***control primer pair*** matching non-allelic sequences.
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.
3 PCR reactions with a reaction volume of 10 µl are performed per sample.
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 3 PCR reactions in an 8 well PCR plate. Wells 4 to 8 are empty.

1	**2**	**3**	**empty**	**empty**	**empty**	**empty**	**empty**

The 8 well PCR plate is marked with 'DRB1*10'.
Tube No. 1 is marked with the Lot No. 'Y61'.
The PCR plates are covered with a PCR-compatible foil.
Please note: When removing each 8 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Only the DRB1*10 alleles will be amplified by the DRB1*10 subtyping kit. Thus, the interpretation of DRB1*10 subtypings is not influenced by other groups of DRB1 alleles or other DRB genes.

UNIQUELY IDENTIFIED ALLELES

All the DRB1*10 alleles, i.e. **DRB1*1001 to DRB1*1002**, recognized by the HLA Nomenclature Committee in January 2008[1] will give rise to unique amplification patterns by the primers in the DRB1*10 subtyping kit.
The DRB1*10 SSP subtyping kit cannot separate the DRB1*100101 and DRB1*100102 alleles.

[1] ***Nomenclature for factors of the HLA system, 1998***. *Tissue Antigens* 1999: **53**: 407-446.
DRB alleles listed on the IMGT/HLA web page 2008-January-11, release 2.20.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 2 DRB1*10 alleles can be combined in 3 homozygous and heterozygous combinations. All these genotypes give rise to unique amplification patterns.

LICENSES

101.129-06 – 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.

101.129-06u – 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.

101.129-06 and 101.129-06u

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 DRB1*10 subtyping kit have the specificities given in the Specificity and Interpretation Tables of the product insert and in the GenoVision version of the HELMBERG-SCORE™ software.
When stored dark 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

DNA EXTRACTION

Extracted, highly pure DNA is needed for SSP typings. We recommend isolation of DNA using GenoPrep B200 or GenoPrep B350 cartridges on the GenoM™-6 robotic workstation (GenoVision Europe *Tel:* +43 1 710 15 00 or GenoVision Inc. USA *Tel:* +1 610 430 88 41; http://www.genovision.com). Using GenoM™-6-extracted DNA ACD, EDTA and heparinised blood can be used as starting material. Because of its high purity, GenoM™-6-extracted DNA can be diluted when used in combination with *Olerup* SSP™ products. The recommended DNA concentration is 15 ng/ul.
Alternatively – BUT DO NOT USE HEPARINISED BLOOD WITH THESE METHODS - the DNA can be extracted using trimethylammoniumbromide salts (DTAB/CTAB) or by salting out. Dissolve the extracted DNA in dH_2O.

IMPORTANT:
Optimal DNA concentration using: GenoM™-6-extracted DNA, 15 ng/µl.
DNA extracted by other methods, 30 ng/µl.

Concentration exceeding 50 ng/µl will increase the risk for nonspecific amplifications and weak extra bands, especially for HLA Class I high resolution SSP typings.

PCR AMPLIFICATION

101.129-06 – licensed for PCR

For one DRB1*10 subtyping, add at room temperature in a 0.5 ml tube:

5 x 2 µl = 10 µl DNA (30 ng/µl)
5 x 3 µl = 15 µl PCR Master Mix complete with *Taq* – mix well before taking your aliquot
5 x 5 µl = 25 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-H_2O mixture into each of the 3 wells of a DRB1*10 subtyping. ***Well No. 1 of the 8 well PCR plate is marked with the lot number.*** Close the 8 well PCR plate with the provided lids.

101.129-06u – <u>not</u> licensed for PCR

For one DRB1*10 subtyping, add at room temperature in a 0.5 ml tube:

5 x 2 µl = 10 µl DNA (30 ng/µl)
5 x 3 µl = 15 µl PCR Master Mix without *Taq* – mix well before taking your aliquot
0.4 µl *Taq* polymerase (5 units/µl)
5 x 5 µl – 0.4 µl = 24.6 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-*Taq*-H_2O mixture into each of the 3 wells of a DRB1*10 subtyping. ***Well No. 1 of the 8 well PCR plate is marked with the lot number.*** Close the 8 well PCR plate with the provided lids.

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. **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 specific PCR products. The relative lengths of the specific PCR products are helpful in the interpretation of the results.

Record the presence and relative lengths of the internal positive control bands. The differently sized control bands will help in the correct orientation of the typing as well as in kit identification.

Lanes without either control band or specific PCR products should be repeated.

Interpret the typings with the ***lot-specific Interpretation and Specificity Tables***.

INTERPRETATION SOFTWARE

The interpretation software (Product No. 110.101) can be helpful in the interpretation of the typings.

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.

SPECIFICITY TABLE

DRB1*10 SSP subtyping

Specificities and sizes of the PCR products of the 3 primer mixes used for DRB1*10 SSP subtyping

Primer Mix	Approx. Size of spec. PCR product[1]	Size of control band[2]	Amplified DRB1*10 alleles
1	210 bp	**515 bp**	100101-100102, 1002
2	205 bp	**515 bp**	100101-100102
3	205 bp	430 bp	1002

[1]Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of DRB1*10 SSP subtypings.
Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.
PCR fragments longer than the control band may sometimes be observed. Such bands can be disregarded and do not influence the interpretation of the SSP typings.
[2]The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs, for most tubes, or a band of 515 base pairs, for some tubes.
Tubes number 1 and 2 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB1*10 subtyping and in order to allow kit identification.

PLEASE NOTE: All the SSP kits, except the B*37, B*41, B*42, B*46, B*47, B*48, B*49, B*50, B*53, B*67, B*78, B*81 and B*82 kits and the Cw*01, Cw*02, Cw*08, Cw*12,Cw*14, Cw*15, Cw*16, Cw*17 and Cw*18 kits, from *Olerup* SSP AB can be uniquely identified by the number of tubes and the kit-specific pattern of the two differently sized control bands.

In the presence of a specific amplification the intensity of the control band often decreases.

INTERPRETATION TABLE			
DRB1*10 SSP subtyping			
Amplification patterns of the DRB1*10 alleles			
	Tube		
	1	**2**	**3**
Length of spec.	**210**	**205**	**205**
PCR product			
Length of int.	**515**	**515**	**430**
pos. control[1]			
5'-primer[2]	**31**	**31**	**31**
	5'-gC g3'	**5'-gC g3'**	**5'-gC g3'**
3'-primer[3]	**87**	**86**	**86**
	5'-CTC3'	**5'-C AC3'**	**5'-C CA3'**
Tube No.	**1**	**2**	**3**
DRB1 allele			
***100101-100102**	**+**	**+**	
***1002**	**+**		**+**
DRB1 allele			
Tube No.	**1**	**2**	**3**

[1]The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs, for most tubes, or a band of 515 base pairs, for some tubes.
Tubes number 1 and 2 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB1*10 subtyping and in order to allow kit identification.

PLEASE NOTE: All the SSP kits, except the B*37, B*41, B*42, B*46, B*47, B*48, B*49, B*50, B*53, B*67, B*78. B*81 and B*82 kits and the Cw*01, Cw*02, Cw*08, Cw*12,Cw*14, Cw*15, Cw*16, Cw*17 and Cw*18 kits, from *Olerup* SSP AB can be uniquely identified by the number of tubes and the kit-specific pattern of the two differently sized control bands.

[2]The codon, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given. Codon numbering as in *Tissue Antigens* 1998, **51:II**, 467-507. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.
[3]The codon, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Codon numbering as in *Tissue Antigens* 1998, **51:II**, 467-507. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.

CELL LINE VALIDATION SHEET

DRB1*10 SSP subtyping kit

					Tube		
					1	2	3
				Production No.	200738901	200738902	200738903
	cell line		DQB1				
1	9001	SA	*0101		-	-	-
2	9280	LK707	*1502	*0405	-	-	-
3	9011	E4181324	*1502		-	-	-
4	9275	GU373	*0301		-	-	-
5	9009	KAS011	*1601		-	-	-
6	9353	SM	*0407	*0803	-	-	-
7	9020	QBL	*0301		-	-	-
8	9007	DEM	*0401	*1602	-	-	-
9	9026	YAR	*0402		-	-	-
10	9107	LKT3	*0405		-	-	-
11	9051	PITOUT	*0701		-	-	-
12	9052	DBB	*0701		-	-	-
13	9067	BTB	*0801		-	-	-
14	9071	OLGA	*0802		-	-	-
15	9075	DKB	*0901		-	-	-
16	9037	SWEIG007	*1101		-	-	-
17	9008	WILJON	*1501		-	-	-
18	9257	32367	*0901	*1101	-	-	-
19	9038	BM16	*1201		-	-	-
20	9059	SLE005	*1302		-	-	-
21	9064	AMALA	*1402		-	-	-
22	9056	KOSE	*1302	*1401	-	-	-
23	9124	IHL	*0803	*1414	-	-	-
24	9035	JBUSH	*1101		-	-	-
25	9049	IBW9	*0701		-	-	-
26	9285	WT49	*0301		-	-	-
27	9191	CH1007	*0405	*1001	+	+	-
28	9320	BEL5GB	*0416	*0701	-	-	-
29	9050	MOU	*0701		-	-	-
30	9021	RSH	*0302		-	-	-
31	9019	DUCAF	*0301		-	-	-
32	9297	HAG	*1303		-	-	-
33	9098	MT14B	*0404		-	-	-
34	9104	DHIF	*1101		-	-	-
35	9302	SSTO	*0403		-	-	-
36	9024	KT17	*0403	*0406	-	-	-
37	9065	HHKB	*1301		-	-	-
38	9099	LZL	*1402		-	-	-
39	9315	CML	*0301	*0401	-	-	-
40	9134	WHONP199	*0701	*0901	-	-	-
41	9055	H0301	*1302		-	-	-
42	9066	TAB089	*0803		-	-	-
43	9076	T7526	*0901		-	-	-
44	9057	TEM	*1401		-	-	-
45	9239	SHJO	*0701		-	-	-
46	9013	SCHU	*1501		-	-	-
47	9045	TUBO	*1104	*1201	-	-	-
48	9303	TER-ND	*0103		-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP™ DRB1*10 SSP

Product number:	**101.129-06 – licensed for PCR** **101.129-06u – not licensed for PCR**
Lot number:	**Y61**
Expiry date:	**2010-February-01**
Number of tests:	**6**
Number of tubes per test:	**3**

Tube specifications:

Tube No.	Production No.
1	2007-389-01
2	2007-389-02
3	2007-389-03

The specificity of each primer solution of the kit has been tested against 48 well characterized cell line DNAs.

No DNAs carrying the allele to be amplified by primer solution 3 were available. The specificities of the primers in primer solution 3 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer.

Results: No false positive or false negative amplifications were obtained.

Date of approval: 2008-February-08

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP™ DRB1*10
Product number: 101.129-06, 101.129-06u
Lot number: Y61

Intended use: DRB1*10 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Hasselstigen 1
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Phone: +46-8-717 88 27
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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 II List B, conformity assessed using Annex IV, 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.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom. (Notified Body number: 0088.)

Saltsjöbaden, Sweden
2008-February-08

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.

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