

Olerup SSP® HLA-Cw*04

Product number:	101.612-12u – without <i>Taq</i> polymerase
Lot number:	06H
Expiry date:	2012-April-01
Number of tests:	12
Number of wells per test:	31
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. 06H.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*® HLA-Cw*04 LOT

The HLA-Cw*04 specificity and interpretation tables have been updated for the HLA-Cw alleles described since the previous *Olerup SSP*® HLA-Cw*04 lot was made (**Lot No. 72F**).

Seven wells has been added to the HLA-Cw*04 kit,
wells **25** to **31**.

The amplification patterns for some rare HLA-Cw*04 alleles
only differ by the length of the specific PCR products.

The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot.

Well	5'-primer	3'-primer	rationale
13	-	Added	Primer added for the Cw*0452 allele.
17	Exchanged	Exchanged	Exchanged primer pair for the Cw*0417 allele, exchanged positive control primer pair.
20	-	Added	Primers added for the Cw*0444 and Cw*0447 alleles.
21	-	Added	Primers added for the Cw*0438 and Cw*0439 alleles.
23	Added	Added	Primer pairs added for the Cw*0440 and Cw*0441 alleles.

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24	Added	-	Primer added for the Cw*0442 allele.
25	New	New	New primer pair for the Cw*0443 allele.
26	New	New	New primer pair for the Cw*0445 allele.
27	New	New	New primer pairs for the Cw*0446 and Cw*0450 alleles.
28	New	New	New primer pair for the Cw*0448 allele.
29	New	New	New primer pair for the Cw*0449 allele.
30	New	New	New primer pair for the Cw*0453 allele.
31	New	New	New primer pair for the Cw*0451 allele.

Change in revision R01 compared to R00:

1. Primer mix 26 may have tendencies of unspecific amplification.

PRODUCT DESCRIPTION

HLA-Cw*04 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-Cw*0401 to HLA-Cw*0453 alleles.

PLATE LAYOUT

Each test consists of 31 PCR reactions in a 32 well PCR plate. Well 32 is empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	empty

The 32 well cut PCR plate is marked with ‘HLA-Cw*04’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘06H’.

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 heat-sealed with a PCR-compatible foil.

Please note: When removing each 32 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

The interpretation of HLA-Cw*04 SSP subtypings will be influenced by other HLA-Cw alleles, as primer mixes 1, 2, 4, 5, 7, 8, 11, 14 and 16 amplify non-HLA-Cw*04 alleles. In addition, the A*2496 allele will be amplified by primer mix 15, the B*5802 allele will be amplified by primer mixes 5 and 8 and the B*0790 allele will be amplified by primer mix 30.

UNIQUELY IDENTIFIED ALLELES

All the HLA-C*04 alleles, i.e. **Cw*0401 to Cw*0453**, recognized by the HLA Nomenclature Committee in January 2010¹ will give rise to unique amplification patterns by the primers in the HLA-Cw*04 subtyping kit.

The HLA-Cw*04 subtyping kit cannot distinguish the Cw*04010101 to Cw*040109 and Cw*040111 to Cw*040115 alleles and the Cw*040401 and Cw*040402 alleles.

The Cw*041502, Cw*0444 and Cw*0447 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 20, the Cw*0423, Cw*0438 and Cw*0439 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 21, the Cw*0425, Cw*0440 and Cw*0441 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 23 and the Cw*0446 and Cw*0450 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 27.

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¹HLA-Cw alleles listed on the IMGT/HLA web page 2010-January-15, release 2.28.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 50 HLA-Cw*04 alleles give rise to 45 different amplification patterns, as the Cw*041502, Cw*0444 and Cw*0447 alleles, the Cw*0423, Cw*0438 and Cw*0439 alleles, the Cw*0425, Cw*0440 and Cw*0441 alleles and the Cw*0446 and Cw*0450 alleles have patterns that only differ in size of specific PCR product. These patterns can be combined in 1035 homozygous and heterozygous combinations. 692 of these do not give rise to unique amplification patterns.

The different lengths of the specific PCR product generated by primer mix 9, 10, 12 to 15, 19 to 21, 23 and 27 were not considered in these calculations.

+++++--+	---+-----	-----	-----	0403,0434 = 0406,0408
+++++---	---+-----	-----	-----	0406,0429 = 0406,0433
+++++---	---+-----	-----	-----	0406,0412 = 0406,0452
+++++---	---+-----	-----	-----	0403,0413 = 0406,0418
+++++---	---+-----	-----	-----	0401,0406 = 040110,0406 = 0403,0404
+++++---	---+-----	-----	-----	0403,0429 = 0403,0433
+++++---	---+-----	-----	-----	0403,0412 = 0403,0452
+++++---	---+-----	-----	-----	0403,0414 = 0403,0428
+++++---	---+-----	-----	-----	0401,0403 = 040110,0403
+++-+---	---+-----	-----	-----	0407,0434 = 0427,0434 = 0432,0434
+++-+---	---+-----	-----	-----	0407,0413 = 0413,0427 = 0413,0432
+++-+---	---+-----	-----	-----	0404,0407 = 0404,0427 = 0404,0432
+++-+---	---+-----	-----	-----	0411,0434 = 0434,0436
+++-+---	---+-----	-----	-----	0410,0434 = 0431,0434
+++-+---	---+-----	-----	-----	0429,0434 = 0433,0434
+++-+---	---+-----	-----	-----	0412,0434 = 0434,0452
+++-+---	---+-----	-----	-----	0408,0413 = 0416,0434 = 0418,0434
+++-+---	---+-----	-----	-----	0426,0434 = 0434,0442
+++-+---	---+-----	-----	-----	0401,0434 = 040110,0434 = 0404,0408 = 0408,0434
+++-+---	---+-----	-----	-----	0411,0413 = 0413,0436
+++-+---	---+-----	-----	-----	0404,0411 = 0404,0436
+++-+---	---+-----	-----	-----	0410,0413 = 0413,0431
+++-+---	---+-----	-----	-----	0404,0410 = 0404,0431
+++-+---	---+-----	-----	-----	0413,0429 = 0413,0433
+++-+---	---+-----	-----	-----	0404,0429 = 0404,0433
+++-+---	---+-----	-----	-----	0412,0413 = 0413,0452
+++-+---	---+-----	-----	-----	0404,0412 = 0404,0452
+++-+---	---+-----	-----	-----	0413,0426 = 0413,0442
+++-+---	---+-----	-----	-----	0401,0413 = 040110,0413 = 0404,0416 = 0404,0418 = 0413,0416 = 0413,0418
+++-+---	---+-----	-----	-----	0404,0426 = 0404,0442
+++-+---	---+-----	-----	-----	0401,0404 = 040110,0404
+++-+---	---+-----	-----	-----	0405,0407 = 0405,0427 = 0405,0432
+++-+---	---+-----	-----	-----	0405,0411 = 0405,0436
+++-+---	---+-----	-----	-----	0405,0410 = 0405,0431
+++-+---	---+-----	-----	-----	0405,0429 = 0405,0433
+++-+---	---+-----	-----	-----	0405,0412 = 0405,0452
+++-+---	---+-----	-----	-----	0405,0416 = 0405,0418
+++-+---	---+-----	-----	-----	0405,0414 = 0405,0428
+++-+---	---+-----	-----	-----	0405,0426 = 0405,0442
+++-+---	---+-----	-----	-----	0401,0405 = 040110,0405 = 0405,0405
+++-+---	---+-----	-----	-----	0407,0408 = 0408,0427 = 0408,0432
+++-+---	---+-----	-----	-----	0407,0435 = 0427,0435 = 0432,0435
+++-+---	---+-----	-----	-----	0407,0430 = 0427,0430 = 0430,0432

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+++---+-	+++-----	-----	-----	0407,0409N = 0409N,0427 = 0409N,0432
+++---+-	+++-----	-----	-----	0407,0411 = 0407,0436 = 0411,0432 = 0427,0436 = 0432,0436
+++---+-	+++-----	-----	-----	0407,0410 = 0407,0431 = 0410,0427 = 0410,0432 = 0427,0431 = 0431,0432
+++---+-	+++-----	-----	-----	0407,0429 = 0407,0433 = 0427,0429 = 0429,0432 = 0432,0433
+++---+-	+++-----	-----	-----	0407,0412 = 0407,0452 = 0412,0427 = 0412,0432 = 0432,0452
+++---+-	+++-----	-----	-----	0407,0418 = 0416,0432 = 0418,0427 = 0418,0432
+++---+-	+++-----	-----	-----	0407,0414 = 0407,0428 = 0414,0427 = 0414,0432 = 0427,0428 = 0428,0432
+++---+-	+++-----	-----	-----	0407,0437 = 0427,0437 = 0432,0437
+++---+-	+++-----	-----	-----	0407,041501 = 041501,0427 = 041501,0432
+++---+-	+++-----	-----	-----	0407,0417 = 0417,0427 = 0417,0432
+++---+-	+++-----	-----	-----	0407,0419 = 0419,0427 = 0419,0432
+++---+-	+++-----	-----	-----	0407,0420 = 0420,0427 = 0420,0432
+++---+-	+++-----	-----	-----	0407,041502 = 041502,0427 = 041502,0432
+++---+-	+++-----	-----	-----	0407,0423 = 0423,0427 = 0423,0432
+++---+-	+++-----	-----	-----	0407,0424 = 0424,0427 = 0424,0432
+++---+-	+++-----	-----	-----	0407,0425 = 0425,0427 = 0425,0432
+++---+-	+++-----	-----	-----	0407,0426 = 0426,0427 = 0426,0432 = 0432,0442
+++---+-	+++-----	-----	-----	0407,0443 = 0427,0443 = 0432,0443
+++---+-	+++-----	-----	-----	0407,0445 = 0427,0445 = 0432,0445
+++---+-	+++-----	-----	-----	0407,0446 = 0427,0446 = 0432,0446
+++---+-	+++-----	-----	-----	0407,0448 = 0427,0448 = 0432,0448
+++---+-	+++-----	-----	-----	0407,0449 = 0427,0449 = 0432,0449
+++---+-	+++-----	-----	-----	0407,0453 = 0427,0453 = 0432,0453
+++---+-	+++-----	-----	-----	0407,0451 = 0427,0451 = 0432,0451
+++---+-	+++-----	-----	-----	0401,0407 = 0401,0427 = 0401,0432 = 040110,0407 = 040110,0432 = 0407,0432 = 0427,0432 = 0432,0432
+++---+-	+++-----	-----	-----	0408,0411 = 0408,0436
+++---+-	+++-----	-----	-----	0408,0410 = 0408,0431
+++---+-	+++-----	-----	-----	0408,0429 = 0408,0433
+++---+-	+++-----	-----	-----	0408,0412 = 0408,0452
+++---+-	+++-----	-----	-----	0408,0416 = 0408,0418
+++---+-	+++-----	-----	-----	0408,0414 = 0408,0428
+++---+-	+++-----	-----	-----	0408,0426 = 0408,0442
+++---+-	+++-----	-----	-----	0401,0408 = 040110,0408 = 0408,0408
+++---+-	+++-----	-----	-----	0411,0435 = 0435,0436
+++---+-	+++-----	-----	-----	0411,0430 = 0430,0436
+++---+-	+++-----	-----	-----	0409N,0411 = 0409N,0436
+++---+-	+++-----	-----	-----	0410,0435 = 0431,0435
+++---+-	+++-----	-----	-----	0410,0430 = 0430,0431
+++---+-	+++-----	-----	-----	0409N,0410 = 0409N,0431
+++---+-	+++-----	-----	-----	0429,0435 = 0433,0435
+++---+-	+++-----	-----	-----	0429,0430 = 0430,0433
+++---+-	+++-----	-----	-----	0409N,0429 = 0409N,0433
+++---+-	+++-----	-----	-----	0412,0435 = 0435,0452
+++---+-	+++-----	-----	-----	0412,0430 = 0430,0452
+++---+-	+++-----	-----	-----	0409N,0412 = 0409N,0452
+++---+-	+++-----	-----	-----	0416,0435 = 0418,0435
+++---+-	+++-----	-----	-----	0416,0430 = 0418,0430
+++---+-	+++-----	-----	-----	0409N,0416 = 0409N,0418
+++---+-	+++-----	-----	-----	0414,0435 = 0428,0435
+++---+-	+++-----	-----	-----	0414,0430 = 0428,0430
+++---+-	+++-----	-----	-----	0409N,0414 = 0409N,0428

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+++----- +--+-----+ --+----- -----	0409N,0437 = 041501,0435 = 0435,0437
+++----- +--+-----+ --+-----+ -----	0420,0430 = 0426,0435 = 0430,0435 = 0435,0442
+++----- +--+-----+ --+----- -----	0401,0435 = 040110,0435 = 0409N,0420 = 0409N,0435 = 0420,0435 = 0435,0435
+++----- +--+-----+ -----+ -----	0401,0430 = 040110,0430 = 0409N,0426 = 0409N,0430 = 0409N,0442 = 0426,0430 = 0430,0430 = 0430,0442
+++----- +--+-----+ -----+ -----	0401,0409N = 040110,0409N = 0409N,0409N
+++----- -++++----- -----	0411,0412 = 0412,0436 = 0436,0452
+++----- -++++-----+ -----	0411,0418 = 0418,0436
+++----- -++++-----+ -----	0411,0414 = 0411,0428 = 0414,0436 = 0428,0436
+++----- -++++-----+ --+----- -----	0411,0437 = 0436,0437
+++----- -++++-----+ -----	0411,041501 = 041501,0436
+++----- -++++-----+ +--+----- -----	0411,0417 = 0417,0436
+++----- -++++-----+ +----- -----	0411,0419 = 0419,0436
+++----- -++++-----+ --+----- -----	0411,0420 = 0420,0436
+++----- -++++-----+ -----+ -----	0411,041502 = 041502,0436
+++----- -++++-----+ -----+ -----	0411,0423 = 0423,0436
+++----- -++++-----+ -----+ -----	0411,0424 = 0424,0436
+++----- -++++-----+ -----+ -----	0411,0425 = 0425,0436
+++----- -++++-----+ -----+ -----	0411,0426 = 0426,0436
+++----- -++++-----+ -----+ +-----	0411,0443 = 0436,0443
+++----- -++++-----+ -----+ +-----	0411,0445 = 0436,0445
+++----- -++++-----+ -----+ +-----	0411,0446 = 0436,0446
+++----- -++++-----+ -----+ +-----	0411,0448 = 0436,0448
+++----- -++++-----+ -----+ +-----	0411,0449 = 0436,0449
+++----- -++++-----+ -----+ +-----	0411,0453 = 0436,0453
+++----- -++++-----+ -----+ +-----	0411,0451 = 0436,0451
+++----- -++++-----+ -----+ +-----	0401,0411 = 0401,0436 = 040110,0436 = 0410,0429 = 0410,0433 = 0411,0429 = 0411,0431 = 0429,0431 = 0429,0436 = 0431,0433 = 0431,0436 = 0433,0436
+++----- -++++-----+ -----+ +-----	0410,0412 = 0410,0452 = 0412,0431 = 0431,0452
+++----- -++++-----+ -----+ +-----	0410,0418 = 0416,0431 = 0418,0431
+++----- -++++-----+ -----+ +-----	0410,0414 = 0410,0428 = 0414,0431 = 0428,0431
+++----- -++++-----+ --+----- -----	0410,0437 = 0431,0437
+++----- -++++-----+ -----+ -----	0410,041501 = 041501,0431
+++----- -++++-----+ +--+----- -----	0410,0417 = 0417,0431
+++----- -++++-----+ +----- -----	0410,0419 = 0419,0431
+++----- -++++-----+ --+----- -----	0410,0420 = 0420,0431
+++----- -++++-----+ -----+ -----	0410,041502 = 041502,0431
+++----- -++++-----+ -----+ -----	0410,0423 = 0423,0431
+++----- -++++-----+ -----+ -----	0410,0424 = 0424,0431
+++----- -++++-----+ -----+ -----	0410,0425 = 0425,0431
+++----- -++++-----+ -----+ -----	0410,0426 = 0426,0431 = 0431,0442
+++----- -++++-----+ -----+ +-----	0410,0443 = 0431,0443
+++----- -++++-----+ -----+ +-----	0410,0445 = 0431,0445
+++----- -++++-----+ -----+ +-----	0410,0446 = 0431,0446
+++----- -++++-----+ -----+ +-----	0410,0448 = 0431,0448
+++----- -++++-----+ -----+ +-----	0410,0449 = 0431,0449
+++----- -++++-----+ -----+ +-----	0410,0453 = 0431,0453
+++----- -++++-----+ -----+ +-----	0410,0451 = 0431,0451
+++----- -++++-----+ -----+ +-----	0401,0410 = 0401,0431 = 040110,0410 = 040110,0431 = 0410,0431 = 0431,0431
+++----- -++++-----+ -----+ +-----	040110,0411 = 0411,0433
+++----- -++++-----+ -----+ +-----	0412,0429 = 0412,0433 = 0429,0452



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+++-----	---+---+---	-----	-----	0416,0429 = 0416,0433 = 0418,0429 = 0418,0433
+++-----	---+---+---	-----	-----	0414,0429 = 0414,0433 = 0428,0429 = 0428,0433
+++-----	---+---+---	---+-----	-----	0429,0437 = 0433,0437
+++-----	---+---+---	-----	-----	041501,0429 = 041501,0433
+++-----	---+---+---	+---+-----	-----	0417,0429 = 0417,0433
+++-----	---+---+---	+-----	-----	0419,0429 = 0419,0433
+++-----	---+---+---	---+-----	-----	0420,0429 = 0420,0433
+++-----	---+---+---	---+-----	-----	041502,0429 = 041502,0433
+++-----	---+---+---	-----+---	-----	0423,0429 = 0423,0433
+++-----	---+---+---	-----+---	-----	0424,0429 = 0424,0433
+++-----	---+---+---	-----+---	-----	0425,0429 = 0425,0433
+++-----	---+---+---	-----+---	-----	0426,0429 = 0426,0433 = 0429,0442 = 0433,0442
+++-----	---+---+---	-----	+-----	0429,0443 = 0433,0443
+++-----	---+---+---	-----	-+-----	0429,0445 = 0433,0445
+++-----	---+---+---	-----	---+-----	0429,0446 = 0433,0446
+++-----	---+---+---	-----	---+-----	0429,0448 = 0433,0448
+++-----	---+---+---	-----	-----+---	0429,0449 = 0433,0449
+++-----	---+---+---	-----	-----+---	0429,0453 = 0433,0453
+++-----	---+---+---	-----	-----+---	0429,0451 = 0433,0451
+++-----	---+---+---	-----	-----	0401,0429 = 0401,0433 = 040110,0429 = 0429,0429 = 0429,0433
+++-----	---+---+---	-----	-----	0412,0416 = 0412,0418 = 0416,0452 = 0418,0452
+++-----	---+---+---	-----	-----	0412,0414 = 0412,0428 = 0414,0452 = 0428,0452
+++-----	---+---+---	---+-----	-----	0412,0437 = 0437,0452
+++-----	---+---+---	-----	-----	0412,041501 = 041501,0452
+++-----	---+---+---	+---+-----	-----	0412,0417 = 0417,0452
+++-----	---+---+---	+-----	-----	0412,0419 = 0419,0452
+++-----	---+---+---	---+-----	-----	0412,0420 = 0420,0452
+++-----	---+---+---	---+-----	-----	0412,041502 = 041502,0452
+++-----	---+---+---	-----+---	-----	0412,0423 = 0423,0452
+++-----	---+---+---	-----+---	-----	0412,0424 = 0424,0452
+++-----	---+---+---	-----+---	-----	0412,0425 = 0425,0452
+++-----	---+---+---	-----+---	-----	0412,0426 = 0412,0442 = 0426,0452 = 0442,0452
+++-----	---+---+---	-----	+-----	0412,0443 = 0443,0452
+++-----	---+---+---	-----	-+-----	0412,0445 = 0445,0452
+++-----	---+---+---	-----	---+-----	0412,0446 = 0446,0452
+++-----	---+---+---	-----	---+-----	0412,0448 = 0448,0452
+++-----	---+---+---	-----	-----+---	0412,0449 = 0449,0452
+++-----	---+---+---	-----	-----+---	0412,0453 = 0452,0453
+++-----	---+---+---	-----	-----+---	0412,0451 = 0451,0452
+++-----	---+---+---	-----	-----	0401,0412 = 0401,0452 = 040110,0412 = 0412,0412 = 0412,0452
+++-----	---+---+---	-----	-----	0414,0416 = 0414,0418 = 0416,0428 = 0418,0428
+++-----	---+---+---	---+-----	-----	0416,0437 = 0418,0437
+++-----	---+---+---	-----	-----	041501,0416 = 041501,0418
+++-----	---+---+---	+---+-----	-----	0416,0417 = 0417,0418
+++-----	---+---+---	+-----	-----	0416,0419 = 0418,0419
+++-----	---+---+---	---+-----	-----	0416,0420 = 0418,0420
+++-----	---+---+---	---+-----	-----	041502,0416 = 041502,0418
+++-----	---+---+---	-----+---	-----	0416,0423 = 0418,0423
+++-----	---+---+---	-----+---	-----	0416,0424 = 0418,0424
+++-----	---+---+---	-----+---	-----	0416,0425 = 0418,0425
+++-----	---+---+---	-----+---	-----	0416,0426 = 0418,0426 = 0418,0442
+++-----	---+---+---	-----	+-----	0416,0443 = 0418,0443
+++-----	---+---+---	-----	-+-----	0416,0445 = 0418,0445

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+++-----	--+----+-	-----	--+-----	0416,0446 = 0418,0446
+++-----	--+----+-	-----	--+-----	0416,0448 = 0418,0448
+++-----	--+----+-	-----	-----+-	0416,0449 = 0418,0449
+++-----	--+----+-	-----	-----+-	0416,0453 = 0418,0453
+++-----	--+----+-	-----	-----+-	0416,0451 = 0418,0451
+++-----	--+----+-	-----	-----+-	0401,0416 = 0401,0418 = 040110,0416 = 040110,0418 = 0416,0418 = 0418,0418
+++-----	--+----+-	-----	-----+-	0414,0437 = 0428,0437
+++-----	--+----+-	-----	-----+-	0414,041501 = 041501,0428
+++-----	--+----+-	+---+-----	-----+-	0414,0417 = 0417,0428
+++-----	--+----+-	-+-----	-----+-	0414,0419 = 0419,0428
+++-----	--+----+-	--+-----	-----+-	0414,0420 = 0420,0428
+++-----	--+----+-	---+-----	-----+-	0414,041502 = 041502,0428
+++-----	--+----+-	-----+---	-----+-	0414,0423 = 0423,0428
+++-----	--+----+-	-----+---	-----+-	0414,0424 = 0424,0428
+++-----	--+----+-	-----+---	-----+-	0414,0425 = 0425,0428
+++-----	--+----+-	-----+---	-----+-	0414,0426 = 0414,0442 = 0426,0428 = 0428,0442
+++-----	--+----+-	-----	+-----	0414,0443 = 0428,0443
+++-----	--+----+-	-----	+-----	0414,0445 = 0428,0445
+++-----	--+----+-	-----	--+-----	0414,0446 = 0428,0446
+++-----	--+----+-	-----	-----+-	0414,0448 = 0428,0448
+++-----	--+----+-	-----	-----+-	0414,0449 = 0428,0449
+++-----	--+----+-	-----	-----+-	0414,0453 = 0428,0453
+++-----	--+----+-	-----	-----+-	0414,0451 = 0428,0451
+++-----	--+----+-	-----	-----+-	0401,0414 = 0401,0428 = 040110,0414 = 040110,0428 = 0414,0428 = 0428,0428
+++-----	--+----+-	--+-----+	-----	0426,0437 = 0437,0442
+++-----	--+----+-	--+-----	-----	0401,0437 = 040110,0437 = 041501,0420 = 041501,0437 = 0420,0437 = 0437,0437
+++-----	--+----+-	-----+	-----	041501,0426 = 041501,0442
+++-----	--+----+-	-----	-----	0401,041501 = 040110,041501 = 041501,041501
+++-----	--+----+-	+---+-----	-----	0417,0426 = 0417,0442
+++-----	--+----+-	+---+-----	-----	0401,0417 = 040110,0417 = 041502,0417 = 0417,0417
+++-----	--+----+-	-+-----+	-----	0419,0426 = 0419,0442
+++-----	--+----+-	-+-----	-----	0401,0419 = 040110,0419 = 0419,0419
+++-----	--+----+-	--+-----+	-----	0420,0426 = 0420,0442
+++-----	--+----+-	--+-----	-----	0401,0420 = 040110,0420 = 0420,0420
+++-----	--+----+-	---+-----+	-----	041502,0426 = 041502,0442
+++-----	--+----+-	---+-----	-----	0401,041502 = 040110,041502 = 041502,041502
+++-----	--+----+-	---+-----+	-----	0423,0426 = 0423,0442
+++-----	--+----+-	---+-----	-----	0401,0423 = 040110,0423 = 0423,0423
+++-----	--+----+-	-----++	-----	0424,0426 = 0424,0442
+++-----	--+----+-	-----+-	-----	0401,0424 = 040110,0424 = 0424,0424
+++-----	--+----+-	-----++	-----	0425,0426 = 0425,0442
+++-----	--+----+-	-----+-	-----	0401,0425 = 040110,0425 = 0425,0425
+++-----	--+----+-	-----+	+-----	0426,0443 = 0442,0443
+++-----	--+----+-	-----+	+-----	0426,0445 = 0442,0445
+++-----	--+----+-	-----+	--+-----	0426,0446 = 0442,0446
+++-----	--+----+-	-----+	-----+-	0426,0448 = 0442,0448
+++-----	--+----+-	-----+	-----+-	0426,0449 = 0442,0449
+++-----	--+----+-	-----+	-----+-	0426,0453 = 0442,0453
+++-----	--+----+-	-----+	-----+-	0426,0451 = 0442,0451
+++-----	--+----+-	-----+	-----+-	0401,0426 = 0401,0442 = 040110,0426 = 040110,0442 = 0426,0426 = 0426,0442



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+++-----	---+-----	-----	+-----	0401,0443 = 040110,0443 = 0443,0443
+++-----	---+-----	-----	-+-----	0401,0445 = 040110,0445 = 0445,0445
+++-----	---+-----	-----	--+-----	0401,0446 = 040110,0446 = 0446,0446
+++-----	---+-----	-----	---+---	0401,0448 = 040110,0448 = 0448,0448
+++-----	---+-----	-----	-----+--	0401,0449 = 040110,0449 = 0449,0449
+++-----	---+-----	-----	-----+-	0401,0453 = 040110,0453 = 0453,0453
+++-----	---+-----	-----	-----++	0401,0451 = 040110,0451 = 0451,0451
+++-----	---+-----	-----	-----	0401,0401 = 0401,040110
+++-----	---+-----	-----	-----	040110,0433 = 0433,0433
+++-----	---+-----	-----	-----	040110,0452 = 0452,0452
++-++-+-	---+-----	-----	-----	0406,0407 = 0406,0427
++-++-+-	---+-----	-----	-----	0403,0407 = 0403,0427
++-++-+-	---+-----	-----	-----	0407,0416 = 0416,0427
++-++-+-	---+-----	-----	-----	0407,0442 = 0427,0442
++-++-+-	---+-----	-----	-----	0407,0407 = 0407,0427
++-++-+-	---+-----	-----	-----	0406,0411 = 0406,0436
++-++-+-	---+-----	-----	-----	0403,0411 = 0403,0436
++-++-+-	---+-----	-----	-----	0411,0416 = 0416,0436
++-++-+-	---+-----	-----	-----	0411,0442 = 0436,0442
++-++-+-	---+-----	-----	-----	0410,0411 = 0410,0436 = 0411,0436 = 0436,0436
---+-----	---+-----	-----	-----	0404,0434 = 0434,0434
---+-----	---+-----	-----	-----	0404,0413 = 0413,0413

0401 = 040101-040109, 040111-040115

0404 = 040401-040402

041502 = 041502, 0444, 0447

0423 = 0423, 0438, 0439

0425 = 0425, 0440, 0441

0446 = 0446, 0450

SPECIFICITY TABLE

HLA-Cw*04 SSP subtyping

Specificities and sizes of the PCR products of the 31 primer mixes used for HLA-Cw*04 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-Cw*04 alleles ³	Other amplified HLA Class I alleles ⁴
1	250 bp	800 bp	*04010101-040115, 0403, 0405, 0407-0412, 041501-0418, 0419 ^w , 0420, 0423-0433, 0435-0453	*010201-0121, 0123-0134, 020201-020203, 020205-020211, 0204-0215, 0217, 0219-0231, 05010101-050111, 0503-0510, 0512-0516, 0518-0526, 0528-0534, 06020101, 06020102, 060203-0603, 0605-0629, 080201-080203, 0805, 0807, 0812, 0815, 0817-0819, 0823, 0825, 0827-0831, 120201-1213, 1215-1217, 1221-1231, 140201-1405, 1407N-1414, 1508, 160401, 1801-1803
2	220 bp	1070 bp	*04010101-040115, 040401-0405, 0407-0409N, 0412 ^w , 0413-041502, 0417-0420, 0423-0435, 0437-0441, 0443-0453	*0764
3 ⁵	145 bp	1070 bp	*04010101-040115, 040401-0405, 0408-041502, 0417-0420, 0423-0426, 0428-0441, 0443-0453	
4	210 bp	1070 bp	*0403, 0406	*0212
5	250 bp	1070 bp	*040401, 040402, 0406, 0413, 0434	*0122, 0203, 021601, 021602, 0218, 0511, 0517, 0527, 0604, 080101, 080102, 080301, 080302, 0804, 0806, 0808-0811, 0813, 0814, 0816, 0820-0822, 0824, 0826N, 121401, 121402, 1218,

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				1220, 1406, 1415, 150201-1507, 1509-1513, 1515-1524, 1526, 17010101-1706, B*5802
6⁵	95 bp	1070 bp	*0405	
7^{5,6}	145 bp	1070 bp	*0407, 0427, 0432	*0334, 0764, 1803
8	270 bp	1070 bp	*0408, 0434	0527, 1515, B*5802
9^{5,8}	110 bp, 180 bp, 220 bp	1070 bp	*0409N, 0430, 0435	
10⁹	195 bp, 220 bp	1070 bp	*0410, 0411, 0431, 0436	
11	180 bp	1070 bp	*04010101-040109, 040111-040115, 0403-0410, 0412- 0420, 0423-0426, 0428-0432, 0434- 0451, 0453	*0525, 0605, 070209, 0828, 1228, 1525
12^{5,10}	125 bp, 165 bp	1070 bp	*0411, 0429, 0433, 0436	
13¹¹	225 bp, 270 bp	1070 bp	*0412, 0452	
14¹²	155 bp, 190 bp	1070 bp	*0413, 0416, 0418	*0708
15^{7,13}	170 bp, 190 bp	1070 bp	*0414, 0428	A*2496
16⁵	130 bp	1070 bp	*041501, 0437	*0305, 0325, 0327, 1409
17⁵	320 bp	1070 bp	*0417	
18	225 bp	1070 bp	*0419	
19^{5,14}	110 bp, 130 bp, 155 bp	1070 bp	*0420, 0435, 0437	
20¹⁵	165 bp, 250 bp, 545 bp	1070 bp	*041502, 0417, 0444, 0447	
21^{5,16}	85 bp, 145 bp, 240 bp	1070 bp	*0423, 0438, 0439	
22⁵	130 bp	1070 bp	*0424	
23^{5,17}	85 bp, 145 bp, 215 bp	1070 bp	*0425, 0440, 0441	
24⁶	160 bp	1070 bp	*0426, 0430, 0442	
25	190 bp	1070 bp	*0443	
26⁷	215 bp	800 bp	*0445	

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27 ^{5,18}	125 bp, 285 bp	800 bp	*0446, 0450	
28	215 bp	1070 bp	*0448	
29	185 bp	1070 bp	*0449	
30 ⁵	75 bp	1070 bp	*0453	B*0790
31	180 bp	1070 bp	*0451	

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 HLA-Cw*04 SSP typings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective lengths of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

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 bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

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 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-Cw*04 subtyping.

In addition, wells number 26 and 27 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

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

³ The Cw*041502, Cw*0444 and Cw*0447 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 20, the Cw*0423, Cw*0438 and Cw*0439 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 21, the Cw*0425, Cw*0440 and Cw*0441 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 23 and the Cw*0446 and Cw*0450 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 27.

⁴ Due to the sharing of sequence motifs between HLA-Cw alleles some non-HLA-Cw*04 alleles will be amplified by primer mixes 1, 2, 4, 5, 7, 8, 11, 14 and 16. In addition, the A*2496 allele will be amplified by primer mix 15, the B*5802 allele will be amplified by primer mixes 5 and 8 and the B*0790 allele will be amplified by primer mix 30.

⁵ Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁶ The primers in primer mix 7 and 24 may give rise to primer oligomer formation.

⁷ Primer mix 26 may have tendencies of unspecific amplification.

⁸ Primer mix 9: Specific PCR fragment of 110 bp in the Cw*0435 allele. Specific PCR fragment of 180 bp in the Cw*0430 allele. Specific PCR fragment of 220 bp in the Cw*0409N allele.

⁹ Primer mix 10: Specific PCR fragment of 195 bp in the Cw*0431 allele. Specific PCR fragment of 220 bp in the Cw*0410, Cw*0411 and Cw*0436 alleles.

¹⁰ Primer mix 12: Specific PCR fragment of 125 bp in the Cw*0429 and Cw*0436 alleles. Specific PCR fragment of 165 bp in the Cw*0433 allele. Specific PCR fragment of 125 and 165 bp in the Cw*0411 allele.

¹¹ Primer mix 13: Specific PCR fragment of 225 bp in the Cw*0412 allele. Specific PCR fragment of 270 bp in the Cw*0452 allele.

¹² Primer mix 14: Specific PCR fragment of 155 bp in the Cw*0416 allele. Specific PCR fragment of 190 bp in the Cw*0413, Cw*0418 and Cw*0708 alleles.

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¹³Primer mix 15: Specific PCR fragment of 170 bp in the Cw*0414 and in the A*2496 alleles. Specific PCR fragment of 190 bp in the Cw*0428 allele.

¹⁴Primer mix 19: Specific PCR fragment of 110 bp in the Cw*0435 allele. Specific PCR fragment of 130 bp in the Cw*0437 allele. Specific PCR fragment of 155 bp in the Cw*0420 allele.

¹⁵Primer mix 20: Specific PCR fragment of 165 bp in the Cw*0444 allele. Specific PCR fragment of 250 bp in the Cw*0447 allele. Specific PCR fragment of 545 bp in the Cw* 041502 and Cw*0417 alleles.

¹⁶Primer mix 21: Specific PCR fragment of 85 bp in the Cw*0423 allele. Specific PCR fragment of 145 bp in the Cw*0438 allele. Specific PCR fragment of 240 bp in the Cw*0439 allele.

¹⁷Primer mix 23: Specific PCR fragment of 85 bp in the Cw*0425 allele. Specific PCR fragment of 145 bp in the Cw*0440 allele. Specific PCR fragment of 215 bp in the Cw*0441 allele.

¹⁸Primer mix 27: Specific PCR fragment of 125 bp in the Cw*0450 allele. Specific PCR fragment of 285 bp in the Cw* 0446 allele.

‘w’, may be weakly amplified.

INTERPRETATION TABLE																
HLA-Cw*04 SSP subtyping																
Amplification patterns of the Cw*0401 to Cw*0453 alleles																
	Well⁹															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length of spec. PCR product	250	220	145	210	250	95	145	270	110	195	180	125	225	155	170	130
									180	220		165	270	190	190	
									220							
Length of int. pos. control¹	800	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070
5'-primer(s)²	2 nd	112	112	118	2 nd	98	112	2 nd	391	112	201	218	112	105	412	368
	5'-CCA [*]	5'-CCT [*]	5'-CCT [*]	5'-CCA [*]	5'-CCA [*]	5'-CTC [*]	5'-CCT [*]	5'-CCA [*]	5'-ACC [*]	5'-CCT [*]	5'-CCA [*]	5'-ggA [*]	5'-CCT [*]	5'-gCT [*]	5'-ATA [*]	5'-gTg [*]
							459		1018	368				368	697	369
							5'-gAT [*]		5'-gTg [*]	5'-gTT [*]				5'-gTT [*]	5'-TCC [*]	5'-TAC [*]
3'-primer(s)³	539	289	218	289	539	154	218	559	459	289	341	302	295	218	539	459
	5'-TCC [*]	5'-AgC [*]	5'-gCT [*]	5'-AgC [*]	5'-TCA [*]	5'-CAg [*]	5'-gCg [*]	5'-CAg [*]	5'-AgA [*]	5'-AgT [*]	5'-CgT [*]	5'-ggC [*]	5'-TCC [*]	5'-gCT [*]	5'-TCT [*]	5'-AgA [*]
		289		289			559		1052	521		341	343	514	846	
		5'-AgC [*]		5'-AgC [*]			5'-CTC [*]		5'-Tgg [*]	5'-ggA [*]		5'-Cgg [*]	5'-T [*]	5'-CTT [*]	5'-CAC [*]	
									1092					527		
									5'-TTA [*]					5'-CCg [*]		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
HLA-Cw allele⁴																
*04010101-040109, 040111-040115	1	2	3								11					
*040110	1	2	3													
*0403	1			4							11					
*040401, 040402		2	3		5						11					
*0405	1	2	3			6					11					
*0406				4	5						11					
*0407	1	2					7				11					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

INTERPRETATION TABLE															
HLA-Cw*04 SSP subtyping															
Amplification patterns of the Cw*0401 to Cw*0453 alleles															
Well ⁹															
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
320	225	110	165	85	130	85	160	190	215	125	215	185	75	180	Length of spec. PCR product
		130	250	145		145				285					
		155	545	240		215									
1070	1070	1070	1070	1070	1070	1070	1070	1070	800	800	1070	1070	1070	1070	Length of int. pos. control ¹
379	368	347	112	368	127	172	89	368	112	368	368	112	503	81	
5'-ACg 5'	5'-gTT 5'	5'-gTA 5'	5'-CCT 5'	5'-gTT 5'	5'-ggA 5'	5'-TCC 5'	5'-gAT 5'	5'-gTT 5'	5'-CCT 5'	5'-gTT 5'	5'-gTT 5'	5'-CCT 5'	5'-CCg 5'	5'-CAG 5'	
		368				355	97								3'-primer(s) ³
		5'-gTg 5'				5'-CCT 5'	5'-TCg 5'								
		391				670	1018								
		5'-ACC 5'				5'-CCg 5'	5'-gTg 5'								
3 rd I	550	459	238	412	218	218	218	518	284	454	544	256	539	218	
5'-ACg 5'	5'-CAT 5'	5'-AgA 5'	5'-CCA 5'	5'-gTC 5'	5'-gCT 5'	5'-gCT 5'	5'-gCT 5'	5'-CCA 5'	5'-gTA 5'	5'-CTg 5'	5'-ggg 5'	5'-CCA 5'	5'-TCC 5'	5'-gCT 5'	
			319	472		459	1052			613					
			5'-gCT 5'	5'-ggC 5'		5'-AgA 5'	5'-Tgg 5'			5'-gCA 5'					
			368	568		846									
			5'-CAT 5'	5'-CTg 5'		5'-CAC 5'									
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Well No. HLA-Cw allele ⁴
															*04010101-040109, 040111-040115
															*040110
															*0403
															*040401, 040402
															*0405
															*0406
															*0407
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Well No.

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Length of spec.	250	220	145	210	250	95	145	270	110	195	180	125	225	155	170	130
PCR product									180	220		165	270	190	190	
									220							
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*0408	1	2	3					8			11					
*0409N	1	2	3						9		11					
*0410	1		3							10	11					
*0411	1		3							10		12				
*0412	1	w	3								11		13			
*0413		2	3		5						11			14		
*0414		2	3								11				15	
*041501	1	2	3								11					16
*041502, 0444, 0447 ⁵	1	2	3								11					
*0416	1										11			14		
*0417	1	2	3								11					
*0418	1	2	3								11			14		
*0419	w	2	3								11					
*0420	1	2	3								11					
*0423, 0438, 0439 ⁶	1	2	3								11					
*0424	1	2	3								11					
*0425, 0440, 0441 ⁷	1	2	3								11					
*0426	1	2	3								11					
*0427	1	2					7									
*0428	1	2	3								11				15	
*0429	1	2	3								11	12				
*0430	1	2	3						9		11					
*0431	1	2	3							10	11					
*0432	1	2	3				7				11					
*0433	1	2	3									12				
*0434		2	3		5			8			11					
*0435	1	2	3						9		11					
*0436	1		3							10	11	12				
*0437	1	2	3								11					16
*0442	1										11					
*0443	1	2	3								11					
*0445	1	2	3								11					
*0446, 0450 ⁸	1	2	3								11					
*0448	1	2	3								11					
*0449	1	2	3								11					
*0451	1	2	3								11					
*0452	1	2	3										13			
*0453	1	2	3								11					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

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320	225	110	165	85	130	85	160	190	215	125	215	185	75	180	Length of spec. PCR product
		130	250	145		145				285					
		155	545	240		215									
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Well No.
															*0408
															*0409N
															*0410
															*0411
															*0412
															*0413
															*0414
															*041501
			20												*041502, 0444, 0447 ⁵
															*0416
17			20												*0417
															*0418
	18														*0419
		19													*0420
				21											*0423, 0438, 0439 ⁶
					22										*0424
						23									*0425, 0440, 0441 ⁷
							24								*0426
															*0427
															*0428
															*0429
							24								*0430
															*0431
															*0432
															*0433
															*0434
		19													*0435
															*0436
		19													*0437
							24								*0442
								25							*0443
									26						*0445
										27					*0446, 0450 ⁸
											28				*0448
												29			*0449
														31	*0451
															*0452
													30		*0453
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Well No.

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Length of spec.	250	220	145	210	250	95	145	270	110	195	180	125	225	155	170	130
PCR product									180	220		165	270	190	190	
									220							
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*010201-0121, 0123-0134, 020201-020203, 020205-020211, 0204-0211, 0213-0215, 0217, 0219-0231, 05010101-050111, 0503-0510, 0512-0516, 0518-0524, 0526, 0528-0534, 06020101, 06020102, 060203-0603, 0606-0629, 080201-080203, 0805, 0807, 0812, 0815, 0817-0819, 0823, 0825, 0827, 0829-0831, 120201-1213, 1215-1217, 1221-1227, 1229-1231, 140201-1405, 1407N, 1408, 1410-1414, 1508, 160401, 1801, 1802	1															
*0122, 0203, 021601, 021602, 0218, 0511, 0517, 0604, 080101, 080102, 080301-0804, 0806, 0808-0811, 0813, 0814, 0816, 0820-0822, 0824, 0826N, 121401, 121402, 1218, 1220, 1406, 1415, 150201-1507, 1509-1513, 1516-1524, 1526, 17010101-1706					5											
*0212	1			4												
*0305, 0325, 0327																16
*0334							7									
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Lot No.: **06H**

Lot-specific information

www.olerup-ssp.com

320	225	110	165	85	130	85	160	190	215	125	215	185	75	180	Length of spec. PCR product
		130	250	145		145				285					
		155	545	240		215									
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Well No.
															*010201-0121, 0123-0134, 020201-020203, 020205-020211, 0204-0211, 0213-0215, 0217, 0219-0231, 05010101-050111, 0503-0510, 0512-0516, 0518-0524, 0526, 0528-0534, 06020101, 06020102, 060203-0603, 0606-0629, 080201-080203, 0805, 0807, 0812, 0815, 0817-0819, 0823, 0825, 0827, 0829-0831, 120201-1213, 1215-1217, 1221-1227, 1229-1231, 140201-1405, 1407N, 1408, 1410-1414, 1508, 160401, 1801, 1802
															*0122, 0203, 021601, 021602, 0218, 0511, 0517, 0604, 080101, 080102, 080301-0804, 0806, 0808-0811, 0813, 0814, 0816, 0820-0822, 0824, 0826N, 121401, 121402, 1218, 1220, 1406, 1415, 150201-1507, 1509-1513, 1516-1524, 1526, 17010101-1706
															*0212
															*0305, 0325, 0327
															*0334
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Well No.

Lot No.: **06H**

Lot-specific information

www.olerup-ssp.com

Length of spec.	250	220	145	210	250	95	145	270	110	195	180	125	225	155	170	130
PCR product									180	220		165	270	190	190	
									220							
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*0525, 0605, 0828, 1228	1										11					
*0527, 1515, B*5802					5			8								
*070209, 1525											11					
*0708														14		
*0764		2					7									
*1409	1															16
*1803	1						7									
HLA-Cw allele ⁴																
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A *2496															15	
B*0790																
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

¹The 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 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-Cw*04 subtyping. .

In addition, wells number 26 and 27 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

²The nucleotide position, in the 2nd, 3rd or 6th, exon or in the 2nd intron, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

³The nucleotide position, in the 2nd, 3rd or 7th exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

⁴The nucleotide sequence of the Cw*0402 allele has been shown to be identical to Cw*04010101. The nucleotide sequence of the Cw*0421 allele has been renamed to Cw*041502.

The nucleotide sequence of the Cw*0422 allele has been shown to be identical to Cw*0421.

⁵The Cw*041502, Cw*0444 and Cw*0447 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 20.

⁶The Cw*0423, Cw*0438 and Cw*0439 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 21.

⁷The Cw*0425, Cw*0440 and Cw*0441 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 23.

⁸The Cw*0446 and Cw*0450 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 27.

Lot No.: **06H**

Lot-specific information

www.olerup-ssp.com

320	225	110	165	85	130	85	160	190	215	125	215	185	75	180	Length of spec. PCR product
		130	250	145		145				285					
		155	545	240		215									
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Well No.
															*0525, 0605, 0828, 1228
															*0527, 1515, B*5802
															*070209, 1525
															*0708
															*0764
															*1409
															*1803
															HLA-Cw allele ⁴
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Well No.
															A*2496
													30		B*0790
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Well No.

⁹Primer mix 9: Specific PCR fragment of 110 bp in the Cw*0435 allele. Specific PCR fragment of 180 bp in the Cw*0430 allele. Specific PCR fragment of 220 bp in the Cw*0409N allele.

Primer mix 10: Specific PCR fragment of 195 bp in the Cw*0431 allele. Specific PCR fragment of 220 bp in the Cw*0410, Cw*0411 and Cw*0436 alleles.

Primer mix 12: Specific PCR fragment of 125 bp in the Cw*0429 and Cw*0436 alleles. Specific PCR fragment of 165 bp in the Cw*0433 allele. Specific PCR fragment of 125 and 165 bp in the Cw*0411 allele.

Primer mix 13: Specific PCR fragment of 225 bp in the Cw*0412 allele. Specific PCR fragment of 270 bp in the Cw*0452 allele.

Primer mix 14: Specific PCR fragment of 155 bp in the Cw*0416 allele. Specific PCR fragment of 190 bp in the Cw*0413, Cw*0418 and Cw*0708 alleles.

Primer mix 15: Specific PCR fragment of 170 bp in the Cw*0414 and in the A*2496 alleles. Specific PCR fragment of 190 bp in the Cw*0428 allele.

Primer mix 19: Specific PCR fragment of 110 bp in the Cw*0435 allele. Specific PCR fragment of 130 bp in the Cw*0437 allele. Specific PCR fragment of 155 bp in the Cw*0420 allele.

Primer mix 20: Specific PCR fragment of 165 bp in the Cw*0444 allele. Specific PCR fragment of 250 bp in the Cw*0447 allele. Specific PCR fragment of 545 bp in the Cw* 041502 and Cw*0417 alleles.

Primer mix 21: Specific PCR fragment of 85 bp in the Cw*0423 allele. Specific PCR fragment of 145 bp in the Cw*0438 allele. Specific PCR fragment of 240 bp in the Cw*0439 allele.

Primer mix 23: Specific PCR fragment of 85 bp in the Cw*0425 allele. Specific PCR fragment of 145 bp in the Cw*0440 allele. Specific PCR fragment of 215 bp in the Cw*0441 allele.

Primer mix 27: Specific PCR fragment of 125 bp in the Cw*0450 allele. Specific PCR fragment of 285 bp in the Cw* 0446 allele.

‘w’, may be weakly amplified.

CELL LINE VALIDATION SHEET					Well															
HLA-Cw*04 SSP subtyping kit																				
				Prod. No.:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
					201070201	201070202	201070203	201070204	201070205	201070206	201070207	201070208	201070209	201070210	201070211	201070212	201070213	201070214	201070215	201070216
IHCW cell line			Cw*																	
1	9001	SA	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*0701	*1505	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*1202		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*0304	*0401	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-
5	9009	KAS011	*0602		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*0304	*0702	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*0501		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025	DEU	*0401		+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-
9	9026	YAR	*1203		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*0102		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0602		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*0102		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*0102	*0304	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*0202		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*0303	*0701	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*0102	*0705	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*0303		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*1203		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*0102	*1502	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*1203		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*0802		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*0704	*1505	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*0501	*1601	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*1701		-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*0501		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*1701	*1703	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*1203		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*0501		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*0303	*0401	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-
37	9065	HHKB	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*0303		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*0202	*0701	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*0602		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*0802		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*0102		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*0102	*0801	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*1203		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*0602	*1701	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*0704	*1502	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*0401	*1601	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-

CELL LINE VALIDATION SHEET																				
HLA-Cw*04 SSP subtyping kit																				
					Well															
					17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
					Prod. No.:	201070217	201070218	201070219	201070220	201070221	201070222	201070223	201070224	201070225	201070226	201070227	201070228	201070229	201070230	201070231
	IHCW cell line		Cw*																	
1	9001	SA	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*0701	*1505	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*1202		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*0304	*0401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*0304	*0702	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*0501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025	DEU	*0401		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*0102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*0102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*0102	*0304	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*0202		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*0303	*0701	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*0102	*0705	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*0303		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*0102	*1502	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*0802		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*0704	*1505	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*0501	*1601	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*1701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*0501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*1701	*1703	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*0501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*0303	*0401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*0303		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*0202	*0701	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*0802		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*0102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*0102	*0801	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*0602	*1701	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*0704	*1502	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*0401	*1601	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-Cw*04 SSP

Product number: 101.612-12u – without *Taq* polymerase
Lot number: 06H
Expiry date: 2012-April-01
Number of tests: 12
Number of wells per test: 31

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2010-702-01	13	2010-702-13	25	2010-702-25
2	2010-702-02	14	2010-702-14	26	2010-702-26
3	2010-702-03	15	2010-702-15	27	2010-702-27
4	2010-702-04	16	2010-702-16	28	2010-702-28
5	2010-702-05	17	2010-702-17	29	2010-702-29
6	2010-702-06	18	2010-702-18	30	2010-702-30
7	2010-702-07	19	2010-702-19	31	2010-702-31
8	2010-702-08	20	2010-702-20		
9	2010-702-09	21	2010-702-21		
10	2010-702-10	22	2010-702-22		
11	2010-702-11	23	2010-702-23		
12	2010-702-12	24	2010-702-24		

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

No DNAs carrying the alleles to be amplified by primer solutions 6, 8, 10, 12, 13, 15 and 17 to 31 were available. The specificity of the primers in primer solutions 8, 10, 12, 13, 15, 17, 20, 21 and 24 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 6, 18 and 25 to 29 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 22, 23, 30 and 31 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 15 and 19 one respective two of the 5'-primers were not possible to test, and in primer solutions 10, 13, 14, 20 and 21 one or two of the 3'-primers were not possible to test. In primer solutions 9 and 24 one 5'-primer and one 3'-primer was not possible to test. Additional primers in primer solutions 7, 9, 14 and 16 were tested by separately adding one 5'-primer and/or one 3'-primer.

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

Date of approval: 2011-July-15

Approved by:

Quality Control, Supervisor

Lot No.: **06H**

Lot-specific information

www.olerup-ssp.com

Declaration of Conformity

Product name: *Olerup* SSP® HLA-Cw*04
Product number: 101.612-12u
Lot number: 06H

Intended use: HLA-Cw*04 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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SE-112 51 Stockholm, Sweden
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 9001:2008 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.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

Stockholm, Sweden
2011-July-15

Olle Olerup
Managing Director

Lot No.: **06H**

Lot-specific information

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