Product Insert

Visit <u>www.olerup-ssp.com</u> for "Instructions for Use" (IFU)

101.123-24/06 – including *Taq* **polymerase**, IFU-01 **101.123-24u/06u – without** *Taq* **polymerase**, IFU-02

Lot No.: **79Y**

Lot-specific information Olerup SSP[®] DRB5

| Product number: | 101.123-24/06 – including <i>Taq</i> pol. 101.123-24u/06u – without <i>Taq</i> pol. |
|----------------------------------|--|
| Lot number: | 79Y |
| Expiry date: | 2018-February-01 |
| Number of tests: | 24 test – Product No. 101.123-24/24u 6 tests – Product No. 101.123-06/06u |
| Number of wells per test: | 15+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. 79Y.

Complete product documentation consists of generic Instructions for Use (IFU), lot specific Product Insert, Worksheet and Certificate.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP[®] DRB5 LOT(49X)

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

The DRB5 specificity and interpretation tables have been updated for the HLA-DRB alleles described since the previous *Olerup* SSP[®] DRB5 lot was made (Lot No. 49X). The kit design is based on IMGT/HLA database 3.21.1.

As of lot series V, the Specificity Table is included in the lot-specific Product Insert, and the Interpretation Table is included in the Worksheet.

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

| Well | 5'-primer | 3'-primer | rationale |
|------|-----------|-----------|---|
| 4 | - | - | Exchanged positive control primer pair for decreased tendency of primer oligomer formation. |

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Lot No.: **79Y**

Lot-specific information

Well **16** contains <u>Negative Control primer pairs</u>, 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 | 105 | 200 | 105 | 80 | 75 | 80 | 85 |
|------------------------|----------------------------------|----------------------------------|----------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| product | | | | | | | |
| 5'-primer ¹ | 164 | 340 | 440 | 45 | 45 | 43 | 36 |
| | ^{5'} -CAC ^{3'} | ^{5'} -Agg ^{3'} | ^{5′} -TTA3' | ^{5'} -Tgg ^{3'} | ^{5'} -Tgg ^{3'} | ^{5'} -Tgg ^{3'} | ^{5'} -TAC ^{3'} |
| | | | | | | | 36 |
| | | | | | | | ^{5'} -TAT ^{3'} |
| 3'-primer ² | 231 | 2 nd I | 507 | 59 | 58 | 57 | 47 |
| - | ⁵ '-TgC ^{3'} | ^{5'} -AAA ^{3'} | ⁵'-TTg³' | ^{5′} -CTC ^{3′} | ^{5'} -ggC ^{3'} | 5'-CTC ^{3'} | ^{5'} -ACA ^{3'} |
| | | | | | | | 48 |
| | | | | | | | ^{5'} -gCA ^{3'} |
| | | | | | | | 48 |
| | | | | | | | ^{5'} -gCC ^{3'} |
| | | | | | | | 52 |
| | | | | | | | ^{5′} -TgT ^{3′} |
| A* | + | + | + | | | | |
| B* | + | + | + | | | | |
| C* | + | + | + | | | | |
| DRB1 | | | | + | ÷ | | |
| DRB3 | | | | + | + | | |
| DRB5 | | | | + | | | |
| DQB1 | | | | | + | | |
| DPB1 | | | | | | + | |
| DQA1 | | | | | | | + |

¹The 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 <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given.

²The 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 <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given. ●LERUP<mark>SSP</mark>® DRB5

Product Insert

101.123-24/06 – including Taq polymerase, IFU-01 **101.123-24u/06u – without Taq polymerase**, IFU-02 Visit <u>www.olerup-ssp.com</u> for "Instructions for Use" (IFU)

Lot No.: **79Y**

Lot-specific information

PRODUCT DESCRIPTION

DRB5 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB5*01:01:01 to DRB5*01:15 and the DRB5*02:02 to DRB5*02:06 alleles.

PLATE LAYOUT

Each test consists of 16 PCR reactions in a 16 well cut PCR plate.

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|----|----|----|----|----|----|----|
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | NC |

The 16 well cut PCR plate is marked with 'DRB5' in silver/gray ink.

Well No. 1 is marked with the Lot No. '79Y'.

Wells 1 to 15 – DRB5 primers.

Well 16 – Negative Control (NC).

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 16 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 alleles of the DRB5 locus will be amplified by the DRB5 subtyping kit, except that the DRB1*09:07 allele will be amplified by primer mixes 1 to 3 and 15.

UNIQUELY IDENTIFIED ALLELES

All the DRB5 alleles, i.e. **DRB5*01:01:01 to DRB5*01:15 and DRB5*02:02 to DRB5*02:06**, recognized by the HLA Nomenclature Committee in August 2015^{1,2} will give rise to unique amplification patterns by the primers in the DRB5 subtyping kit.

¹DRB5 alleles listed on the IMGT/HLA web page 2015-August-11, release 3.21.1, <u>www.ebi.ac.uk/imgt/hla</u>.

²Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page <u>http://hla.alleles.org/alleles/deleted.html</u>.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DRB5 homo- and heterozygotes is available upon request.



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"Instructions for Use" (IFU)

101.123-24/06 – including *Taq* **polymerase**, IFU-01 **101.123-24u/06u – without** *Taq* **polymerase**, IFU-02

Lot No.: **79Y**

Lot-specific information SPECIFICITY TABLE

DRB5 SSP subtyping

Specificities and sizes of the PCR products of the 15+1 primer mixes used for DRB5 SSP subtyping

| Primer Mix | Size of spec. PCR product ¹ | Size of control band ² | Amplified DRB5 alleles ³ | Other amplified DRB alleles ⁴ |
|-----------------|--|---|---|---|
| 1 | 255 bp | 515 bp | *01:01:01-01:05, 01:07- 01:15, 02:03 | DRB1*09:07 |
| 2 | 210 bp | 515 bp | *01:01:01-01:05, 01:07- 01:10N, 01:12-01:15, 02:04 | DRB1*09:07 |
| 3 ⁶ | 225 bp | 430 bp | *01:01:01-01:02, 01:04- 01:05, 01:07-01:10N, 01:12-01:15, 02:05 | DRB1*09:07 |
| 4 ⁵ | 100 bp 150 bp | 515 bp | *01:01:01-01:01:02, 01:04, 01:06-01:07, 01:09, 01:11, 01:15 *02:06 | |
| 5 | 150 bp | 515 bp | *01:01:01, 01:05, 01:07, 01:09, 01:13 | |
| 6 | 145 bp | 430 bp | *01:02-01:03, 01:05, 01:08N, 01:10N | |
| 7 | 145 bp | 430 bp | *01:02-01:03, 01:08N, 01:10N, 02:05 | |
| 8 | 215 bp | 430 bp | *01:03, 01:06, 01:11, 02:02-02:04, 02:06 | |
| | | | | |
| 9 | 175 bp 225 bp | 430 bp | *01:13 *01:04 | |
| 10 | 130 bp 160 bp | 430 bp | *01:07 *01:12, 01:15 | |
| 11 | 200 bp | 430 bp | *01:06, 01:11, 02:02-02:03, 02:06 | |
| 12 | 185 bp | 515 bp | *02:02, 02:04-02:06 | |
| 13 | 195 bp | 430 bp | *01:08N | |
| 14 ⁵ | 110 bp 210 bp | 430 bp | *01:14 *01:09 | |
| 15 | 235 bp | 430 bp | *01:10N, 01:12, 01:15 | DRB1*09:07 |
| 16 ⁷ | - | - | Negative Control | |

¹Alleles 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 DRB*07 SSP subtypings. When the primers in a primer mix can give rise to HLA-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits, the alleles listed are specified according to amplicon length.



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Lot No.: 79Y

Lot-specific information

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 inherit 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.

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the longer, 515 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases. In the presence of a specific amplification the intensity of the control band often decreases.

³For several DRB alleles 1st and/or 3rd exon(s) and above, as well as intron nucleotide sequences, are not available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. Assumption is made that unknown sequences in these regions are conserved within allelic groups and that unknown sequences of codons 87 to 92 are identical with the DRB1*01:01 consensus sequence.

⁴Due to the sharing of sequence motifs between DRB alleles the DRB1*09:07 allele will be amplified by primer mixes 1 to 3 and 15.

⁵HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁶Primer mix 3 frequently gives rise to an extra band longer than the control band. This band should be disregarded in the interpretation of DRB5 SSP typings.

⁷Primer mix 16 contains a negative control, which will amplify more than 95% of HLA amplicons as well as 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 and the PCR product generated by the HGH positive control primer pair is 430 base pairs.



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Lot No.: **79Y**

●LERUPSSP

Lot-specific information PRIMER SPECIFICATION

| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Length of spec. | 255 | 210 | 225 | 100 | 150 | 145 | 145 | 215 |
| PCR product(s) | | | | 150 | | | | |
| | | | | | | | | |
| Length of int. | 515 | 515 | 430 | 515 | 515 | 430 | 430 | 430 |
| pos. control ¹ | | | | | | | | |
| 5'-primer(s) ² | 13(125) | 13(125) | 13(125) | 37(199) | 36(196) | 37(199) | 36(196) | 13(125) |
| | ^{5'} -gTA ^{3'} | ^{5'} -gTA ^{3'} | ^{5'} -gTA ^{3'} | 5' -ACT 3' | ^{5'} -Agg ^{3'} | ^{5'} -ACg ^{3'} | ^{5'} -AgA ^{3'} | ^{5'} -gTA ^{3'} |
| | | | | 97(379) | | 37(199) | | |
| | | | | ^{5'} -CTg ^{3'} | | ^{5'} -ACg ^{3'} | | |
| 3'-primer(s) ³ | 85(341) | 66(286) | 71(299) | 57(258) | 72(303) | 72(303) | 69(295) | 71(299) |
| | | | | ^{5'} -gCg ^{3'} | | | | |
| | | | | 134(490) | | | | 71(299) |
| | | | | ^{5'} -gCC ^{3'} | | | ^{5'} -gCg ^{3'} | |
| | | 70(296) | 77(319) | | | | | |
| | | 5' -TCC 3' | 5' -CAC 3' | | | | | |
| | | 72(303) | | | | | | |
| | | ^{5'} -gCg ^{3'} | | | | | | |
| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

| Well No. | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Length of spec. | 175 | 130 | 200 | 185 | 195 | 110 | 235 |
| PCR product(s) | 225 | 160 | | | | 210 | |
| | | | | | | | |
| Length of int. | 430 | 430 | 430 | 515 | 430 | 430 | 430 |
| pos. control ¹ | | | | | | | |
| 5'-primer(s) ² | 13(125) | • • | | | 107(409) | | |
| | ^{5'} -gTA ^{3'} | ^{5'} -ACT ^{3'} | ^{5'} -gTA ^{3'} | ^{5'} -AgA ^{3'} | ^{5'} -AgA ^{3'} | ^{5'} -gTA ^{3'} | ^{5'} -gTA ^{3'} |
| 3'-primer(s) ³ | 58(260) | 66(286) | 66(286) | 85(341) | 159(565) | 36(196) | 77(319) |
| | 5' -CCT 3' | ^{5'} -gAT ^{3'} | ^{5'} -gAT ^{3'} | ^{5'} -CAg ^{3'} | ^{5'} -CAT ^{3'} | ^{5'} -gTA ^{3'} | ^{5'} -CAC ^{3'} |
| | 73(307) | 77(319) | | | | 69(295) | 79(323) |
| | ^{5'} -CAg ^{3'} | ^{5'} -CAC ^{3'} | | | | ^{5'} -gTT ^{3'} | ^{5'} -TgC ^{3'} |
| Well No. | 9 | 10 | 11 | 12 | 13 | 14 | 15 |

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the longer, 515 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

²The nucleotide position matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given.

³The nucleotide position matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence or the 3 terminal nucleotides of the primer is given.

Lot No.: 79Y

Lot-specific information

101.123-24/06 – including *Taq* polymerase, IFU-01 101.123-24u/06u – without *Taq* polymerase, IFU-02

| | CELL LINE VALIDATION SHEET | | | | | | | | | | | | | | | | | |
|--------------|----------------------------|--------------------------|------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|----------------|-----------|-----------|-----------|-----------|-----------|-----------|
| | | | DRB5 \$ | | | | | | | | | | • | | | | | |
| | | | | | | | | | 5 | | /el | 1 ² | | | | | | |
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| | | | | - | 2 | <i>с</i> | 4 | ß | 9 | ~ | 8 | 6 | 0 | - | 2 | <i>с</i> | 4 | ß |
| | | | Prod. No.: | 201556401 | 201556402 | 201556403 | 201556404 | 201556405 | 201556406 | 201556407 | 201556408 | 201556409 | 201556410 | 201556411 | 201556412 | 201556413 | 201556414 | 201556415 |
| | | | d. 1 | 155 | 155 | 155 | 155 | 155 | 155 | 155 | 155 | 155 | 155 | 155 | 155 | 155 | 155 | 155 |
| | | | Pro | 20 | 20 | 20 | 20 | 20, | 20, | 20 | 20 | 20, | 20, | 20, | 20 | 20 | 20, | 20, |
| | IHW | C cell line ¹ | DRB5 | | | | | | | | | | | | | | | |
| 1 | 9001 | | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | | LK707 | *01:02 | + | + | + | - | - | + | + | - | - | - | - | - | - | - | - |
| 3 | | E4181324 | *01:02 | + | + | + | - | - | + | + | - | - | - | - | - | - | - | - |
| 4 | | GU373 | | - | • | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | | KAS011 | *02:02 | - | - | - | - | - | - | - | + | - | - | + | + | - | - | - |
| 6 7 | 9353 9020 | | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 8 | 9020 | | _ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 9 | | YAR | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 10 | | LKT3 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 11 | | PITOUT | | - | • | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 12 | 9052 | | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 13 | | JESTHOM | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 14 | | OLGA | | - | • | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 15 | 9075 | | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 16 | | SWEIG007 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 17 18 | | CTM3953540 32367 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 10 | | BM16 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 20 | | SLE005 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 21 | | AMALA | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 22 | | KOSE | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 23 | 9124 | IHL | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 24 | | JBUSH | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 25 | | IBW9 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 26 | | WT49 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 27 | | CH1007 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 28 29 | | BEL5GB MOU | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 30 | 9030 | | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 31 | | DUCAF | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 32 | | HAG | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 33 | | MT14B | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | • |
| 34 | 9104 | | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 35 | | SSTO | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 36 | | KT17 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 37 | | HHKB | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 38 39 | 9099 9315 | | | - | - | - | - | - | - | - | - | - | - | - | • | - | - | - |
| - 39 - 40 | | WHONP199 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 41 | | H0301 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 42 | | TAB089 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 43 | | T7526 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 44 | 9057 | TEM | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 45 | | SHJO | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 46 | | SCHU | *01:01 | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - |
| 47 | | TUBO | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 48 | 9303 | TER-ND | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |



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Lot-specific information

¹The provided cell line HLA specificities are retrieved from the <u>http://www.ihwg.org/hla</u> web site. The specificity of an individual cell line may thus be subject to change.

²The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 9, 10, 14 and 15 were available. The specificities of the primers in primer solutions 9, 10, 14 and 15 were tested by separately adding one or two additional 5'-primer, respectively one or two additional 3'-primer(s). In primer solution 4 one 5'-primer was not possible to test, and in primer solutions 2, 14 and 15 one 3'-primer was not possible to test. Additional primers in primer solutions 1 to 4, 7, 8 and 11 were tested by separately adding additional 5'-primers or 3'-primers.



Lot No.: **79Y**

Lot-specific information



Lot No.: **79Y**

Lot-specific information



Lot No.: **79Y**

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Visit <u>www.olerup-ssp.com</u> for "Instructions for Use" (IFU)

Lot No.: **79Y**

Lot-specific information

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For information on Olerup SSP distributors worldwide, contact Olerup GmbH.

