# *Olerup* SSP® HLA-B*49

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| **Product number:** | **101.547-06 – including *Taq* polymerase** |
| **Lot number:** | **74M** |
| **Expiry date:** | **2014-June-01** |
| **Number of tests:** | **6** |
| **Number of wells per test:** | **16** |
| **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. 74M.

## CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® HLA-B*49 LOT.

The HLA-B*49 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup* SSP® HLA-B*49 lot was made **(Lot No. 86K)**.

The amplification patterns for some rare HLA-B*49 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 |
|---|---|---|---|
| 7 | - | Added | Primer added for the B*49:20 allele. |
| 8 | - | Added | Primer added for the B*49:19N allele. |
| 10 | - | Added | Primer added for the B*49:16 allele. |
| 14 | - | Added | Primers added for the B*49:17 and B*49:19N alleles. |
| 15 | - | Added | Primer added for the B*49:17 allele. |
| 16 | Added | Added | Primer pair added for the B*49:18 allele. |

# PRODUCT DESCRIPTION

## HLA-B*49 SSP typing

### CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*49:01 to B*49:20 alleles.

### PLATE LAYOUT

Each HLA-B*49 test consists of 16 PCR reactions in a 16 well cut PCR plate.

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|---|---|---|---|---|---|---|---|
| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |

The 16 well PCR plate is marked with 'HLA-B*49' in silver/gray ink.
Well No. 1 is marked with the Lot No. '74M'.
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 16 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-B*49 SSP subtypings will be influenced by two B*07, four B*08, most B*13, several B*15, two B*18, three B*27, six B*35, the B*37:10, the B*38:30, nine B*39, several B*40, nine B*41, the B*42:04, most B*44, the B*45, two B*46, the B*47, the B*50, most B*51, the B*52, the B*53:22, most B*54, most B*55, most B*56, four B*57, three B*58, the B*59, the B*73 and the B*78 alleles when present on the other haplotype.

### UNIQUELY IDENTIFIED ALLELES

All the HLA-B*49, i.e. **B*49:01 to B*49:20**, recognized by the HLA Nomenclature Committee in October 2011[1] will be amplified by the primers in the HLA-B*49 SSP kit.
The HLA-B*49 subtyping kit cannot distinguish the B*49:01:01 to B*49:01:03 alleles.
The B*49:08 and 49:16 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 10.
The B*49:13 and 49:14 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 15.

[1]HLA-B alleles listed on the IMGT/HLA web page 2011-October-10, release 3.6.0, www.ebi.ac.uk/imgt/hla.

## RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 22 alleles generate 18 amplification patterns that can be combined in 171 homozygous and heterozygous combinations. 115 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products were not considered in these calculations.

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| +++−+++− −−−−−−−− | *49:02, *49:04 = *49:02, *49:20 |
| +++−++−− −−−−−−−+ | *49:02, *49:15 = *49:02, *49:18 |
| +++−++−− −−−−−−−− | *49:01:01, *49:02 = *49:02, *49:06 |
| ++−+++−− −−−−−−−− | *49:03, *49:06 = *49:06, *49:09 |
| ++−−+++− −−−−−−−− | *49:04, *49:06 = *49:06, *49:20 |
| ++−−++−− −−−−−−−+ | *49:06, *49:15 = *49:06, *49:18 |
| ++−−++−− −−−−−−−− | *49:01:01, *49:06 = *49:06, *49:06 |
| +−−++++ −−−−−−−− | *49:03, *49:05 = *49:05, *49:09 |
| +−−+++− −−−−−−−− | *49:03, *49:04 = *49:03, *49:20 = *49:04, *49:09 = *49:09, *49:20 |
| +−−+++−+ −−−−−+−− | *49:03, *49:19N = *49:09, *49:19N |
| +−−++−− +−−−−−−− | *49:03, *49:07 = *49:07, *49:09 |
| +−−++−− −+−−−−−− | *49:03, *49:08 = *49:08, *49:09 |
| +−−++−− −−−+−−−− | *49:03, *49:10 = *49:09, *49:10 |
| +−−++−− −−−−+−−− | *49:03, *49:11 = *49:09, *49:11 |
| +−−++−− −−−−−++− | *49:03, *49:17 = *49:09, *49:17 |
| +−−++−− −−−−−+−− | *49:03, *49:12 = *49:09, *49:12 |
| +−−++−− −−−−−−+− | *49:03, *49:13 = *49:09, *49:13 |
| +−−++−− −−−−−−−+ | *49:03, *49:15 = *49:03, *49:18 = *49:09, *49:15 = *49:09, *49:18 |
| +−−++−− −−−−−−−− | *49:01:01, *49:03 = *49:01:01, *49:09 = *49:03, *49:09 = *49:09, *49:09 |
| +−−−++++ −−−−−+−− | *49:04, *49:19N = *49:05, *49:12 = *49:05, *49:19N = *49:19N, *49:20 |
| +−−−++++ −−−−−−−− | *49:01:01, *49:05 = *49:05, *49:20 |
| +−−−+++− +−−−−−−− | *49:04, *49:07 = *49:07, *49:20 |
| +−−−+++− −+−−−−−− | *49:04, *49:08 = *49:08, *49:20 |
| +−−−+++− −−−+−−−− | *49:04, *49:10 = *49:10, *49:20 |
| +−−−+++− −−−−+−−− | *49:04, *49:11 = *49:11, *49:20 |
| +−−−+++− −−−−−++− | *49:04, *49:17 = *49:17, *49:20 |
| +−−−+++− −−−−−+−− | *49:04, *49:12 = *49:12, *49:20 |
| +−−−+++− −−−−−−+− | *49:04, *49:13 = *49:13, *49:20 |
| +−−−+++− −−−−−−−+ | *49:04, *49:15 = *49:15, *49:20 = *49:18, *49:20 |
| +−−−+++− −−−−−−−− | *49:01:01, *49:04 = *49:01:01, *49:20 = *49:04, *49:20 = *49:20, *49:20 |
| +−−−++−+ −−−−−++− | *49:13, *49:19N = *49:17, *49:19N |
| +−−−++−+ −−−−−+−+ | *49:15, *49:19N = *49:18, *49:19N |
| +−−−++−+ −−−−−+−− | *49:01:01, *49:19N = *49:12, *49:19N = *49:19N, *49:19N |
| +−−−++−− +−−−−−−+ | *49:07, *49:15 = *49:07, *49:18 |
| +−−−++−− +−−−−−−− | *49:01:01, *49:07 = *49:07, *49:07 |
| +−−−++−− −+−−−−−+ | *49:08, *49:15 = *49:08, *49:18 |
| +−−−++−− −+−−−−−− | *49:01:01, *49:08 = *49:08, *49:08 |
| +−−−++−− −−−+−−−+ | *49:10, *49:15 = *49:10, *49:18 |
| +−−−++−− −−−+−−−− | *49:01:01, *49:10 = *49:10, *49:10 |
| +−−−++−− −−−−+−−+ | *49:11, *49:15 = *49:11, *49:18 |
| +−−−++−− −−−−+−−− | *49:01:01, *49:11 = *49:11, *49:11 |

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|---|---|
| +−−−++−− −−−−−+++ | *49:15, *49:17 = *49:17, *49:18 |
| +−−−++−− −−−−−++− | *49:01:01, *49:17 = *49:12, *49:13 = *49:12, *49:17 = *49:13, *49:17 = *49:17, *49:17 |
| +−−−++−− −−−−−+−+ | *49:12, *49:15 = *49:12, *49:18 |
| +−−−++−− −−−−−+−− | *49:01:01, *49:12 = *49:12, *49:12 |
| +−−−++−− −−−−−++ | *49:13, *49:15 = *49:13, *49:18 |
| +−−−++−− −−−−−+− | *49:01:01, *49:13 = *49:13, *49:13 |
| +−−−++−− −−−−−−+ | *49:01:01, *49:15 = *49:01:01, *49:18 = *49:15, *49:15 = *49:15, *49:18 |
| +−−−−−++ −−−−−−− | *49:04, *49:05 = *49:05, *49:05 |

*49:01:01 = *49:01:01-49:01:03
*49:08 = *49:08 and 49:16
*49:13 = *49:13 and *49:14

# SPECIFICITY TABLE

## HLA-B*49 SSP subtyping

**Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-B*49 SSP subtyping**

| Primer Mix | Size of spec. PCR product[1] | Size of control band[2] | Amplified HLA-B*49 alleles[3] | Other amplified HLA-B alleles[4] |
|---|---|---|---|---|
| **1** | 145 bp | **800 bp** | *49:01:01-49:01:03, 49:04-49:20 | *40:13, 40:19, 40:109, 40:117, 44:18, 44:25, 44:50, 44:95, 51:112, 57:45, 57:51 |
| **2**[6,8] | 145 bp, 255 bp | 1070 bp | *49:02, 49:06 | *13:23, 18:09, 27:01, 37:10, 40:47, 40:96, 40:157, 44:15, 44:55, 44:103, 58:13 |
| **3** | 145 bp | 1070 bp | *49:02 | *40:47, 40:96, 40:110, 40:157, 44:02:01:01-44:02:12, 44:02:14-44:02:18, 44:02:20-44:05:02, 44:07, 44:10, 44:12-44:17, 44:19N-44:24, 44:26-44:39, 44:41:01-44:43:02, 44:45, 44:47-44:49, 44:51-44:56N, 44:58N-44:59, 44:61N-44:74, 44:76-44:82, 44:84-44:89, 44:91-44:94, 44:96-44:128, 44:132-44:133, 44:135-44:137, 47:04 |
| **4**[9] | 170 bp, 205 bp | 1070 bp | *49:03, 49:09 | *13:15, 18:04, 40:01:11, 40:58, 44:02:10, 44:34, 51:07:01, 51:62, 51:106, 52:01:02, 52:01:04, 52:01:09, 52:02-52:03, 52:09, 52:21, 52:25 |
| **5**[5] | 105 bp | 1070 bp | *49:01:01-49:03, 49:06-49:17, 49:19N-49:20 | *07:78, 13:02:01-13:03, 13:08Q-13:09, 13:14-13:16, 13:18-13:19, 13:27, 13:30-13:34, 13:37-13:38, 13:40-13:42, 13:44-13:45, 13:48-13:49N, 15:42, 35:60, 44:15, 44:18, 45:01, 45:03-45:08, 45:10-45:13, 46:11, 46:18, 50:01:01-50:02, 50:04-50:08, 50:10-50:11, 50:13, 50:15, 51:15, 52:25, 54:01:01-54:03, 54:05N, 54:07-54:08N, 54:10, 54:12-54:13, 54:16-54:24, 55:01:01-55:01:06, 55:01:08-55:03, 55:05, 55:07, 55:09-55:12, 55:15-55:16, 55:18-55:19, 55:21-55:22, 55:24-55:26, 55:29-55:31, 55:33-55:38, 55:40-55:41, 55:43, 55:45-55:48, 55:50, 55:52, 55:54, 56:01:01-56:01:04, 56:07-56:08, 56:13-56:14, 56:16-56:17, 56:19N-56:20:02, 56:23-56:30, 59:01:01:01-59:01:01:02, 59:04-59:05 |
| **6**[7] | 160 bp | 1070 bp | *49:01:01-49:03, 49:06-49:17, | *13:01:01-13:03, 13:06-13:09, 13:11-13:12, 13:14-13:17, 13:19-13:20, 13:22:01-13:23, 13:25, 13:27-13:30, 13:32-13:34, 13:36-13:40, 13:42-13:45, 13:47-13:50, 13:52, |

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| | | | 49:19N-49:20 | 15:42, 15:86, 15:224, 35:60, 39:17, 40:48, 40:71, 44:10, 46:11, 46:18, 50:01:01-50:02, 50:04-50:08, 50:10-50:13, 50:15, 51:15, 51:62, 51:106, 52:25, 54:01:01-54:03, 54:05N, 54:07-54:08N, 54:12-54:13, 54:17-54:19, 54:21-54:24, 55:01:01-55:03, 55:05, 55:07, 55:10-55:12, 55:15-55:16, 55:18-55:19, 55:22, 55:24-55:26, 55:29-55:31, 55:33-55:36, 55:38-55:45, 55:47-55:48, 55:50, 55:53-55:54, 56:01:01-56:02, 56:04, 56:07-56:08, 56:10, 56:14, 56:16-56:17, 56:19N-56:20:02, 56:23-56:30, 59:01:01:01-59:01:01:02, 59:04-59:05 |
| **7**[5,10] | 105 bp, 255 bp | 1070 bp | *49:04-49:05, 49:20 | *13:04, 13:46, 15:04, 15:16:01-15:16:03, 15:67, 15:95, 15:137, 15:155, 15:222, 35:37, 39:06:01-39:06:02, 39:33-39:34, 39:50, 39:57, 39:62, 39:64, 40:86, 40:159, 44:15, 44:18, 44:20, 44:47, 44:100, 45:01-45:03, 45:05-45:07, 45:09, 45:11-45:13, 50:02, 50:09, 51:13:01-51:13:02, 51:23, 51:37, 51:63, 51:92, 51:97, 51:108, 52:14-52:15, 54:14, 55:13, 55:23, 55:32, 56:22, 59:03, 73:01-73:02 |
| **8**[5,11] | 70 bp, 155 bp | 1070 bp | *49:05, 49:19N | *07:78, 07:84, 08:09, 13:02:01-13:02:05, 13:02:07-13:04, 13:08Q-13:09, 13:14-13:16, 13:18-13:19, 13:27, 13:30-13:35, 13:37-13:38, 13:40-13:42, 13:44-13:49N, 15:04, 15:16:01-15:16:02, 15:42, 15:67, 15:83, 15:95, 15:137, 15:155, 15:222, 27:14, 27:81, 35:60, 39:06:01-39:06:02, 39:33-39:34, 39:50, 39:57, 39:62, 39:64, 40:06:01:01-40:06:04, 40:44, 40:53, 40:70, 40:75, 40:83, 40:86, 40:93, 40:95-40:96, 40:103, 40:109-40:110, 40:127, 40:131, 40:148, 40:159, 40:161-40:162, 40:165, 40:177, 41:01, 41:05-41:07, 41:09, 41:12, 41:14, 41:16-41:17, 42:04, 44:20, 44:47, 44:100, 46:11, 46:18, 51:01:01-51:01:18, 51:01:20-51:03, 51:05, 51:07:01-51:16, 51:18-51:24:04, 51:26-51:41N, 51:43-51:44N, 51:48-51:55, 51:57-51:58, 51:60-51:61, 51:63, 51:65-51:80, 51:82-51:93, 51:95-51:126, 52:01:01:01-52:27, 54:01:01-54:05N, 54:07-54:08N, 54:10-54:14, 54:16-54:24, 55:01:01-55:01:06, 55:01:08-55:03, 55:05, 55:07, 55:09-55:13, 55:15-55:26, 55:28-55:48, 55:50, 55:52-55:54, 56:01:01-56:01:04, 56:05:01-56:08, 56:13-56:17, |

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| | | | | 56:19N-56:30, 58:08:01-58:08:02, 59:01:01:01-59:05, 73:01-73:02, 78:01:01-78:07 |
| | | | | |
| **9** | 245 bp | 1070 bp | *49:07 | *07:78, 07:84, 13:02:01-13:02:12, 13:08Q-13:09, 13:14-13:16, 13:18-13:19, 13:27, 13:30-13:35, 13:37-13:38, 13:40-13:42, 13:44-13:45, 13:47, 13:49N, 27:14, 27:81, 39:50, 40:06:01:01-40:06:04, 40:44, 40:53, 40:70, 40:75, 40:83, 40:86, 40:93, 40:95-40:96, 40:103, 40:109-40:110, 40:127, 40:131, 40:148, 40:159, 40:161-40:162, 40:165, 40:167, 40:177, 45:10, 51:10, 51:16, 51:31, 51:34, 51:82, 51:93, 52:08, 55:09, 55:22, 55:24, 73:01-73:02 |
| **10**[5,12] | 80 bp, 250 bp | **800 bp** | *49:08, 49:16 | |
| **11** | 505 bp | 1070 bp | | *08:03, 08:52, 08:78, 38:30, 44:06, 44:18, 44:25, 44:95, 51:08, 51:20, 51:36, 51:44N, 51:97, 52:19, 53:22, 57:09, 57:24 |
| **12** | 135 bp | 1070 bp | *49:10 | *15:46, 15:53, 15:106, 35:47, 35:154, 40:10:01-40:10:02, 40:40, 40:49, 40:58, 40:110, 40:129, 40:164, 44:02:01:01-44:05:03, 44:09-44:14, 44:16-44:17, 44:19N-44:39, 44:41:01-44:43:02, 44:45-44:48, 44:50-44:54, 44:56N, 44:58N-44:59, 44:61N-44:80, 44:82, 44:84-44:102, 44:104-44:110, 44:112-44:129, 44:132-44:133, 44:135-44:137, 47:01:01:01-47:08, 50:13, 57:45 |
| **13**[7] | 150 bp | 1070 bp | *49:11 | *13:01:01-13:03, 13:06-13:09, 13:11-13:12, 13:14-13:15, 13:17, 13:18[w], 13:19, 13:22:01-13:23, 13:25, 13:27-13:30, 13:32-13:34, 13:36-13:40, 13:42-13:45, 13:47, 13:49N-13:50, 13:52, 15:86, 15:224, 35:60, 40:48, 40:71, 44:10, 46:11, 46:18, 51:15, 54:01:01-54:03, 54:05N, 54:07-54:08N, 54:10[w], 54:12-54:13, 54:17-54:19, 54:21-54:24, 55:02:01-55:02:06, 55:07, 55:10, 55:12, 55:16, 55:18-55:19, 55:22, 55:26, 55:30, 55:34-55:35, 55:37[w], 55:39, 55:41-55:43, 55:47-55:48, 55:50, 56:01:01-56:02, 56:04, 56:07-56:08, 56:10, 56:14, 56:16-56:17, 56:19N-56:20:02, 56:23-56:24, 56:26-56:29, 59:01:01:01-59:01:01:02, 59:04-59:05 |

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|---|---|---|---|---|
| **14**[13] | 155 bp, 190 bp | 1070 bp | *49:12, 49:17, 49:19N | |
| **15**[7,14] | 160 bp, 190 bp, 290 bp | 1070 bp | *49:13-49:14, 49:17 | |
| **16**[5,15] | 95 bp, 135 bp | 1070 bp | *49:15, 49:18 | *35:01:10, 35:04:02, 40:28, 50:14, 51:56:01-51:56:02, 55:01:04 |

[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-B*49 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 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.
[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-B*49 SSP subtyping. In addition, well number 10 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 B*49:08 and 49:16 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 10.
The B*49:13 and 49:14 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 15.
[4]Due to the sharing of sequence motifs between HLA-B alleles some non-HLA-B*49 alleles will be amplified by primer mixes 1 to 9, 11 to 13 and 16.
[5]Short specific PCR fragments are less intense and not as sharp as longer specific bands.
[6]Primer mix 2 may generate a false band of about 800 base pairs. This band should be disregarded when interpreting the HLA-B*49 SSP typings.
[7]Primer mixes 6, 13 and 15 have a tendency of giving rise to nonspecific amplifications.
[8]Primer mix 2: Specific PCR fragment of 145 bp in the B*49:06 and the B*58:13 alleles. Specific PCR fragment of 255 bp in the B*49:02 and in the B*13:23, 18:09, 27:01, 37:10, 40:47, 40:96, 40:157, 44:15, 44:55, 44:103 alleles.
[9]Primer mix 4: Specific PCR fragment of 170 bp in the B*49:03 and in the B*18:04, 40:01:11, 40:58, 44:02:10, 44:34, 51:07:01, 52:01:02, 52:01:04, 52:01:09, 52:02-52:03, 52:09 and 52:21 alleles. Specific PCR fragment of 205 bp in the B*49:09 and in the B*13:15, 51:62, 51:106 and 52:25 alleles.
[10]Primer mix 7: Specific PCR fragment of 105 bp in the B*49:04 and 49:05 and in the B*13:04, 13:46, 15:04, 15:16:01-15:16:03, 15:67, 15:95, 15:137, 15:155, 15:222, 35:37, 39:06:01-39:06:02, 39:33-39:34, 39:50, 39:57, 39:62, 39:64, 40:86, 40:159, 45:02, 45:09, 50:09, 51:13:01-51:13:02, 51:37, 51:63, 51:92, 51:97, 52:14, 54:14, 55:13, 55:23, 55:32, 56:22, 59:03 and 73:01-73:02 alleles. Specific PCR fragment of 255 bp in the B*49:20 and in the B*44:15,

44:18, 44:20, 44:47, 44:100, 45:01-45:03, 45:05-45:07, 45:09, 45:11-45:13, 50:02, 51:23, 51:108 and 52:15 alleles.

[11]Primer mix 8: Specific PCR fragment of 70 bp in the B*49:05 and in the B*07:78, 07:84, 08:09, 13:02:01-13:02:05, 13:02:07-13:04, 13:08Q-13:09, 13:14-13:16, 13:18-13:19, 13:27, 13:30-13:35, 13:37-13:38, 13:40-13:42, 13:44-13:49N, 15:04, 15:16:01-15:16:02, 15:42, 15:67, 15:83, 15:95, 15:137, 15:155, 15:222, 27:14, 27:81, 35:60, 39:06:01-39:06:02, 39:33-39:34, 39:50, 39:57, 39:62, 39:64, 40:06:01:01-40:06:04, 40:44, 40:53, 40:70, 40:75, 40:83, 40:86, 40:93, 40:95-40:96, 40:103, 40:109-40:110, 40:127, 40:131, 40:148, 40:159, 40:161-40:162, 40:165, 40:177, 41:01, 41:05-41:07, 41:09, 41:12, 41:14, 41:16-41:17, 42:04, 44:20, 44:47, 44:100, 46:11, 46:18, 51:01:01-51:01:18, 51:01:20-51:03, 51:05, 51:07:01-51:16, 51:18-51:24:04, 51:26-51:41N, 51:43-51:44N, 51:48-51:55, 51:57-51:58, 51:60-51:61, 51:63, 51:65-51:80, 51:82-51:93, 51:95-51:126, 52:01:01:01-52:27, 54:01:01-54:05N, 54:07-54:08N, 54:10-54:14, 54:16-54:24, 55:01:01-55:01:06, 55:01:08-55:03, 55:05, 55:07, 55:09-55:13, 55:15-55:26, 55:28-55:48, 55:50, 55:52-55:54, 56:01:01-56:01:04, 56:05:01-56:08, 56:13-56:17, 56:19N-56:30, 58:08:01-58:08:02, 59:01:01:01-59:05, 73:01-73:02 and 78:01:01-78:07 alleles. Specific PCR fragment of 155 bp in the B*49:19N allele.

[12]Primer mix 10: Specific PCR fragment of 80 bp in the B*49:16 allele. Specific PCR fragment of 250 bp in the B*49:08 allele.

[13]Primer mix 14: Specific PCR fragment of 155 bp in the B*49:19N allele. Specific PCR fragment of 190 bp in the B*49:12 and 49:17 alleles.

[14]Primer mix 15: Specific PCR fragment of 160 bp in the B*49:13 allele. Specific PCR fragment of 190 bp in the B*49:17 allele. Specific PCR fragment of 290 bp in the B*49:14 allele.

[15]Primer mix 16: Specific PCR fragment of 95 bp in the B*49:18 and in the B*35:01:10, 35:04:02, 40:28, 50:14, 51:56:01-51:56:02 and 55:01:04 alleles. Specific PCR fragment of 135 bp in the B*49:15 allele.

'w', may be weakly amplified.

# INTERPRETATION TABLE

## HLA-B*49 SSP subtyping

### Amplification patterns of the B*49:01 to B*49:20 alleles

| | Well[6] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Length of spec. | 145 | 145 | 145 | 170 | 105 | 160 | 105 | 70 |
| PCR product(s) | | 255 | | 205 | | | 255 | 155 |
| | | | | | | | | |
| Length of int. | | | | | | | | |
| pos. control[1] | 800 | 1070 | 1070 | 1070 | 1070 | 1070 | 1070 | 1070 |
| 5'-primer[2] | 206 | 97 | 206 | 141 | 357 | 420 | 357 | 357 |
| | 5' -gAA 3' | 5' -TCC 3' | 5' -gAA 3' | 5' -ATT 3' | 5' -Tgg 3' | 5' -TTA 3' | 5' -Tgg 3' | 5' -Tgg 3' |
| | | 454 | | 420 | | | | |
| | | 5' -ACA 3' | | 5' -TTA 3' | | | | |
| | | | | | | | | |
| 3'-primer(s)[3] | 309 | 309 | 309 | 272 | 420 | 538 | 419 | 387 |
| | 5' -ATC 3' | 5' -gTg 3' | 5' -gTg 3' | 5' -Tgg 3' | 5' -gCT 3' | 5' -CAg 3' | 5' -Cgg 3' | 5' -TCC 3' |
| | | 559 | | 583 | | | 419 | 470 |
| | | 5' -CAg 3' | | 5' -gTg 3' | | | 5' -CgA 3' | 5' -TCT 3' |
| | | | | | | | 572 | |
| | | | | | | | 5' -gCg 3' | |
| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| HLA-B allele | | | | | | | | |
| *49:01:01-49:01:03 | 1 | | | | 5 | 6 | | |
| *49:02 | | 2 | 3 | | 5 | 6 | | |
| *49:03 | | | | 4 | 5 | 6 | | |
| *49:04 | 1 | | | | | | 7 | |
| *49:05 | 1 | | | | | | 7 | 8 |
| *49:06 | 1 | 2 | | | 5 | 6 | | |
| *49:07 | 1 | | | | 5 | 6 | | |
| *49:08, 49:16[4] | 1 | | | | 5 | 6 | | |
| *49:09 | 1 | | | 4 | 5 | 6 | | |
| *49:10 | 1 | | | | 5 | 6 | | |
| *49:11 | 1 | | | | 5 | 6 | | |
| *49:12 | 1 | | | | 5 | 6 | | |
| *49:13, 49:14[5] | 1 | | | | 5 | 6 | | |
| *49:15 | 1 | | | | 5 | 6 | | |
| *49:17 | 1 | | | | 5 | 6 | | |
| *49:18 | 1 | | | | | | | |
| *49:19N | 1 | | | | 5 | 6 | | 8 |
| *49:20 | 1 | | | | 5 | 6 | 7 | |
| *07:78, 13:31, 13:41, 55:09 | | | | | 5 | | | 8 |
| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

# INTERPRETATION TABLE

## HLA-B*49 SSP subtyping

### Amplification patterns of the B*49:01 to B*49:20 alleles

| Well[6] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | |
| 245 | 80 | 505 | 135 | 150 | 155 | 160 | 95 | Length of spec. |
| | 250 | | | | 190 | 190 | 135 | PCR product(s) |
| | | | | | | 290 | | |
| | | | | | | | | Length of int. |
| 1070 | 800 | 1070 | 1070 | 1070 | 1070 | 1070 | 1070 | pos. control[1] |
| 357 | 357 | 317 | 97 | 420 | 357 | 357 | 176 | 5'-primer[2] |
| 5' -Tgg 3' | 5' -Tgg 3' | 5' -gCT 3' | 5' -TCT 3' | 5' -TTA 3' | 5' -Tgg 3' | 5' -Tgg 3' | 5' -gAC 3' | |
| | | | | | | | 379 | |
| | | | | | | | 5' -ACC 3' | |
| 559 | 394 | 538 | 193 | 527 | 470 | 476 | 272 | 3'-primer(s)[3] |
| 5' -CTC 3' | 5' -gCA 3' | 5' -gTC 3' | 5' -CgT 3' | 5' -CCA 3' | 5' -TCT 3' | 5' -CCC 3' | 5' -Tgg 3' | |
| | 565 | | | | 505 | 506 | 435 | |
| | 5' -CAg 3' | | | | 5' -gCT 3' | 5' -Tgg 3' | 5' -TCT 3' | |
| | | | | | 506 | 608 | | |
| | | | | | 5' -Tgg 3' | 5' -gCT 3' | | |
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Well No. |
| | | | | | | | | HLA-B allele |
| | | | | | | | | *49:01:01-49:01:03 |
| | | | | | | | | *49:02 |
| | | | | | | | | *49:03 |
| | | | | | | | | *49:04 |
| | | | | | | | | *49:05 |
| | | | | | | | | *49:06 |
| 9 | | | | | | | | *49:07 |
| | 10 | | | | | | | *49:08, 49:16[4] |
| | | | | | | | | *49:09 |
| | | | 12 | | | | | *49:10 |
| | | | | 13 | | | | *49:11 |
| | | | | | 14 | | | *49:12 |
| | | | | | | 15 | | *49:13, 49:14[5] |
| | | | | | | | 16 | *49:15 |
| | | | | | 14 | 15 | | *49:17 |
| | | | | | | | 16 | *49:18 |
| | | | | | 14 | | | *49:19N |
| | | | | | | | | *49:20 |
| 9 | | | | | | | | *07:78, 13:31, 13:41, 55:09 |
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Well No. |

| **Length of spec.** | **145** | **145** | **145** | **170** | **105** | **160** | **105** | **70** |
|---|---|---|---|---|---|---|---|---|
| **PCR product(s)** | | **255** | | **205** | | | **255** | **155** |
| | | | | | | | | |
| **Well No.** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| ***07:84, 13:35, 27:14, 27:81, 40:06:01:01-40:06:04, 40:44, 40:53, 40:70, 40:75, 40:83, 40:93, 40:95, 40:103, 40:127, 40:131, 40:148, 40:161-40:162, 40:165, 40:177, 51:10, 51:16, 51:31, 51:34, 51:82, 51:93, 52:08** | | | | | | | | **8** |
| ***08:03, 08:52, 08:78, 38:30, 44:06, 53:22, 57:09, 57:24** | | | | | | | | |
| ***08:09, 15:83, 41:01, 41:05-41:07, 41:09, 41:12, 41:14, 41:16-41:17, 42:04, 51:01:01-51:01:18, 51:01:20-51:03, 51:05, 51:07:02, 51:09:01-51:09:02, 51:11N-51:12, 51:14, 51:18-51:19, 51:21-51:22, 51:24:01-51:24:04, 51:26-51:30, 51:32-51:33, 51:35, 51:38-51:41N, 51:43, 51:48-51:55, 51:57-51:58, 51:60-51:61, 51:65-51:80, 51:83-51:91, 51:95-51:96, 51:98N-51:105, 51:107, 51:109-51:111, 51:113-51:126, 52:01:01:01-52:01:01:02, 52:01:03, 52:01:05-52:01:08, 52:01:10, 52:04-52:07, 52:10:01-52:13, 52:16-52:18, 52:20, 52:22-52:24, 52:26-52:27, 54:04, 54:11, 55:17, 55:20, 55:28, 56:05:01-56:06, 56:15, 56:21, 58:08:01-58:08:02, 59:02, 78:01:01-78:07** | | | | | | | | **8** |
| ***13:01:01-13:01:06, 13:06-13:07N, 13:11-13:12, 13:17, 13:22:01-13:22:02, 13:25, 13:28-13:29, 13:36, 13:39, 13:43, 13:50, 13:52, 15:86, 15:224, 40:48, 40:71, 56:02, 56:04, 56:10** | | | | | | **6** | | |
| **Well No.** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |

| 245 | 80 | 505 | 135 | 150 | 155 | 160 | 95 | Length of spec. |
|---|---|---|---|---|---|---|---|---|
| | 250 | | | | 190 | 190 | 135 | PCR product(s) |
| | | | | | | 290 | | |
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Well No. |
| 9 | | | | | | | | *07:84, 13:35, 27:14, 27:81, 40:06:01:01-40:06:04, 40:44, 40:53, 40:70, 40:75, 40:83, 40:93, 40:95, 40:103, 40:127, 40:131, 40:148, 40:161-40:162, 40:165, 40:177, 51:10, 51:16, 51:31, 51:34, 51:82, 51:93, 52:08 |
| | | 11 | | | | | | *08:03, 08:52, 08:78, 38:30, 44:06, 53:22, 57:09, 57:24 |
| | | | | | | | | *08:09, 15:83, 41:01, 41:05-41:07, 41:09, 41:12, 41:14, 41:16-41:17, 42:04, 51:01:01-51:01:18, 51:01:20-51:03, 51:05, 51:07:02, 51:09:01-51:09:02, 51:11N-51:12, 51:14, 51:18-51:19, 51:21-51:22, 51:24:01-51:24:04, 51:26-51:30, 51:32-51:33, 51:35, 51:38-51:41N, 51:43, 51:48-51:55, 51:57-51:58, 51:60-51:61, 51:65-51:80, 51:83-51:91, 51:95-51:96, 51:98N-51:105, 51:107, 51:109-51:111, 51:113-51:126, 52:01:01:01-52:01:01:02, 52:01:03, 52:01:05-52:01:08, 52:01:10, 52:04-52:07, 52:10:01-52:13, 52:16-52:18, 52:20, 52:22-52:24, 52:26-52:27, 54:04, 54:11, 55:17, 55:20, 55:28, 56:05:01-56:06, 56:15, 56:21, 58:08:01-58:08:02, 59:02, 78:01:01-78:07 |
| | | | | 13 | | | | *13:01:01-13:01:06, 13:06-13:07N, 13:11-13:12, 13:17, 13:22:01-13:22:02, 13:25, 13:28-13:29, 13:36, 13:39, 13:43, 13:50, 13:52, 15:86, 15:224, 40:48, 40:71, 56:02, 56:04, 56:10 |
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Well No. |

| Length of spec. | 145 | 145 | 145 | 170 | 105 | 160 | 105 | 70 |
|---|---|---|---|---|---|---|---|---|
| PCR product(s) | | 255 | | 205 | | | 255 | 155 |
| | | | | | | | | |
| **Well No.** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| *13:02:01-13:02:05, 13:02:07-13:02:12, 13:08Q-13:09, 13:14, 13:19, 13:27, 13:30, 13:32-13:34, 13:37-13:38, 13:40, 13:42, 13:44-13:45, 13:49N, 55:22 | | | | | 5 | 6 | | 8 |
| *13:02:06 | | | | | 5 | 6 | | |
| *13:03, 35:60, 46:11, 46:18, 51:15, 54:01:01-54:03, 54:05N, 54:07-54:08N, 54:12-54:13, 54:17-54:19, 54:21-54:24, 55:02:01-55:02:06, 55:07, 55:10, 55:12, 55:16, 55:18-55:19, 55:26, 55:30, 55:34-55:35, 55:41, 55:43, 55:47-55:48, 55:50, 56:01:01-56:01:04, 56:07-56:08, 56:14, 56:16-56:17, 56:19N-56:20:02, 56:23-56:24, 56:26-56:29, 59:01:01:01-59:01:01:02, 59:04-59:05 | | | | | 5 | 6 | | 8 |
| *13:04, 13:46, 15:04, 15:16:01-15:16:02, 15:67, 15:95, 15:137, 15:155, 15:222, 39:06:01-39:06:02, 39:33-39:34, 39:57, 39:62, 39:64, 51:13:01-51:13:02, 51:23, 51:37, 51:63, 51:92, 51:108, 52:14-52:15, 54:14, 55:13, 55:23, 55:32, 56:22, 59:03 | | | | | | | 7 | 8 |
| *13:15 | | | | 4 | 5 | 6 | | 8 |
| *13:16, 55:24 | | | | | 5 | 6 | | 8 |
| *13:18 | | | | | 5 | | | 8 |
| *13:20, 39:17, 50:12, 55:01:07 | | | | | | 6 | | |
| *13:23 | | 2 | | | | 6 | | |
| *13:47 | | | | | | 6 | | 8 |
| *13:48, 15:42, 55:01:01-55:01:03, 55:01:05-55:01:06, 55:01:08, 55:03, 55:05, 55:11, 55:15, 55:25, 55:29, 55:31, 55:33, 55:36, 55:38, 55:40, 55:45, 55:54, 56:25, 56:30 | | | | | 5 | 6 | | 8 |
| *15:16:03, 35:37, 45:02, 45:09, 50:09 | | | | | | | 7 | |
| **Well No.** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |

| 245 | 80 | 505 | 135 | 150 | 155 | 160 | 95 | Length of spec. |
|---|---|---|---|---|---|---|---|---|
| | 250 | | | | 190 | 190 | 135 | PCR product(s) |
| | | | | | | 290 | | |
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Well No. |
| 9 | | | | 13 | | | | *13:02:01-13:02:05, 13:02:07-13:02:12, 13:08Q-13:09, 13:14, 13:19, 13:27, 13:30, 13:32-13:34, 13:37-13:38, 13:40, 13:42, 13:44-13:45, 13:49N, 55:22 |
| 9 | | | | 13 | | | | *13:02:06 |
| | | | | 13 | | | | *13:03, 35:60, 46:11, 46:18, 51:15, 54:01:01-54:03, 54:05N, 54:07-54:08N, 54:12-54:13, 54:17-54:19, 54:21-54:24, 55:02:01-55:02:06, 55:07, 55:10, 55:12, 55:16, 55:18-55:19, 55:26, 55:30, 55:34-55:35, 55:41, 55:43, 55:47-55:48, 55:50, 56:01:01-56:01:04, 56:07-56:08, 56:14, 56:16-56:17, 56:19N-56:20:02, 56:23-56:24, 56:26-56:29, 59:01:01:01-59:01:01:02, 59:04-59:05 |
| | | | | | | | | *13:04, 13:46, 15:04, 15:16:01-15:16:02, 15:67, 15:95, 15:137, 15:155, 15:222, 39:06:01-39:06:02, 39:33-39:34, 39:57, 39:62, 39:64, 51:13:01-51:13:02, 51:23, 51:37, 51:63, 51:92, 51:108, 52:14-52:15, 54:14, 55:13, 55:23, 55:32, 56:22, 59:03 |
| 9 | | | | 13 | | | | *13:15 |
| 9 | | | | | | | | *13:16, 55:24 |
| 9 | | | | w | | | | *13:18 |
| | | | | | | | | *13:20, 39:17, 50:12, 55:01:07 |
| | | | | 13 | | | | *13:23 |
| 9 | | | | 13 | | | | *13:47 |
| | | | | | | | | *13:48, 15:42, 55:01:01-55:01:03, 55:01:05-55:01:06, 55:01:08, 55:03, 55:05, 55:11, 55:15, 55:25, 55:29, 55:31, 55:33, 55:36, 55:38, 55:40, 55:45, 55:54, 56:25, 56:30 |
| | | | | | | | | *15:16:03, 35:37, 45:02, 45:09, 50:09 |
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Well No. |

| Length of spec. | 145 | 145 | 145 | 170 | 105 | 160 | 105 | 70 |
|---|---|---|---|---|---|---|---|---|
| PCR product(s) | | 255 | | 205 | | | 255 | 155 |
| | | | | | | | | |
| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| *15:46, 15:53, 15:106, 35:47, 35:154, 40:10:01-40:10:02, 40:40, 40:49, 40:129, 40:164, 44:02:13, 44:02:19, 44:05:03, 44:09, 44:11, 44:46, 44:75, 44:90, 44:129, 47:01:01:01-47:03, 47:05-47:08 | | | | | | | | |
| *18:04, 40:01:11 | | | | 4 | | | | |
| *18:09, 27:01, 37:10, 58:13 | | 2 | | | | | | |
| *35:01:10, 35:04:02, 40:28, 50:14, 51:56:01-51:56:02 | | | | | | | | |
| *39:50, 40:86, 40:159, 73:01-73:02 | | | | | | | 7 | 8 |
| *40:13, 40:19, 40:117, 57:51 | 1 | | | | | | | |
| *40:47, 40:157, 44:55, 44:103 | | 2 | 3 | | | | | |
| *40:58 | | | | 4 | | | | |
| *40:96 | | 2 | 3 | | | | | 8 |
| *40:109 | 1 | | | | | | | 8 |
| *40:110 | | | 3 | | | | | 8 |
| *40:167 | | | | | | | | |
| *44:02:01:01-44:02:09, 44:02:11-44:02:12, 44:02:14-44:02:18, 44:02:20-44:05:02, 44:12-44:14, 44:16-44:17, 44:19N, 44:21-44:24, 44:26-44:33, 44:35-44:39, 44:41:01-44:43:02, 44:45, 44:48, 44:51-44:54, 44:56N, 44:58N-44:59, 44:61N-44:74, 44:76-44:80, 44:82, 44:84-44:89, 44:91-44:94, 44:96-44:99, 44:101-44:102, 44:104-44:110, 44:112-44:128, 44:132-44:133, 44:135-44:137, 47:04 | | | 3 | | | | | |
| *44:02:10, 44:34 | | | 3 | 4 | | | | |
| *44:07, 44:49, 44:81, 44:111 | | | 3 | | | | | |
| *44:10 | | | 3 | | | 6 | | |
| *44:15 | | 2 | 3 | | 5 | | 7 | |
| *44:18 | 1 | | | | 5 | | 7 | |
| *44:20, 44:47, 44:100 | | | 3 | | | | 7 | 8 |
| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

| 245 | 80 | 505 | 135 | 150 | 155 | 160 | 95 | Length of spec. |
|---|---|---|---|---|---|---|---|---|
| | 250 | | | | 190 | 190 | 135 | PCR product(s) |
| | | | | | | 290 | | |
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Well No. |
| | | | 12 | | | | | *15:46, 15:53, 15:106, 35:47, 35:154, 40:10:01-40:10:02, 40:40, 40:49, 40:129, 40:164, 44:02:13, 44:02:19, 44:05:03, 44:09, 44:11, 44:46, 44:75, 44:90, 44:129, 47:01:01:01-47:03, 47:05-47:08 |
| | | | | | | | | *18:04, 40:01:11 |
| | | | | | | | | *18:09, 27:01, 37:10, 58:13 |
| | | | | | | | 16 | *35:01:10, 35:04:02, 40:28, 50:14, 51:56:01-51:56:02 |
| 9 | | | | | | | | *39:50, 40:86, 40:159, 73:01-73:02 |
| | | | | | | | | *40:13, 40:19, 40:117, 57:51 |
| | | | | | | | | *40:47, 40:157, 44:55, 44:103 |
| | | | 12 | | | | | *40:58 |
| 9 | | | | | | | | *40:96 |
| 9 | | | | | | | | *40:109 |
| 9 | | | 12 | | | | | *40:110 |
| 9 | | | | | | | | *40:167 |
| | | | 12 | | | | | *44:02:01:01-44:02:09, 44:02:11-44:02:12, 44:02:14-44:02:18, 44:02:20-44:05:02, 44:12-44:14, 44:16-44:17, 44:19N, 44:21-44:24, 44:26-44:33, 44:35-44:39, 44:41:01-44:43:02, 44:45, 44:48, 44:51-44:54, 44:56N, 44:58N-44:59, 44:61N-44:74, 44:76-44:80, 44:82, 44:84-44:89, 44:91-44:94, 44:96-44:99, 44:101-44:102, 44:104-44:110, 44:112-44:128, 44:132-44:133, 44:135-44:137, 47:04 |
| | | | 12 | | | | | *44:02:10, 44:34 |
| | | | | | | | | *44:07, 44:49, 44:81, 44:111 |
| | | | 12 | 13 | | | | *44:10 |
| | | | | | | | | *44:15 |
| | | 11 | | | | | | *44:18 |
| | | | 12 | | | | | *44:20, 44:47, 44:100 |
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Well No. |

| **Length of spec.** | **145** | **145** | **145** | **170** | **105** | **160** | **105** | **70** |
|---|---|---|---|---|---|---|---|---|
| **PCR product(s)** | | **255** | | **205** | | | **255** | **155** |
| | | | | | | | | |
| **Well No.** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| ***44:25, 44:95** | **1** | | | | | | | |
| ***44:50, 57:45** | **1** | | | | | | | |
| ***45:01, 45:03, 45:05-45:07, 45:11-45:13** | | | | | **5** | | **7** | |
| ***45:04, 45:08** | | | | | **5** | | | |
| ***45:10** | | | | | **5** | | | |
| ***50:01:01-50:01:03, 50:04-50:08, 50:10-50:11, 50:15** | | | | | **5** | **6** | | |
| ***50:02** | | | | | **5** | **6** | **7** | |
| ***50:13** | | | | | **5** | **6** | | |
| ***51:07:01, 52:01:02, 52:01:04, 52:01:09, 52:02-52:03, 52:09, 52:21** | | | | **4** | | | | **8** |
| ***51:08, 51:20, 51:36, 51:44N, 52:19** | | | | | | | | **8** |
| ***51:62** | | | | **4** | | **6** | | |
| ***51:97** | | | | | | | **7** | **8** |
| ***51:106** | | | | **4** | | **6** | | **8** |
| ***51:112** | **1** | | | | | | | **8** |
| ***52:25** | | | | **4** | **5** | **6** | | **8** |
| ***54:10, 55:37** | | | | | **5** | | | **8** |
| ***54:16, 54:20, 55:21, 55:46, 55:52, 56:13** | | | | | **5** | | | **8** |
| ***55:01:04** | | | | | **5** | **6** | | **8** |
| ***55:39, 55:42** | | | | | | **6** | | **8** |
| ***55:44, 55:53** | | | | | | **6** | | **8** |
| **HLA-B allele** | | | | | | | | |
| **Well No.** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |

[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 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-B*49 SSP subtyping.
In addition, well number 10 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.
[2]The nucleotide position, in the 2nd or 3rd exon, 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.
[3]The nucleotide position, in the 2nd or 3rd 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.

| 245 | 80 | 505 | 135 | 150 | 155 | 160 | 95 | Length of spec. |
|---|---|---|---|---|---|---|---|---|
| | 250 | | | | 190 | 190 | 135 | PCR product(s) |
| | | | | | | 290 | | |
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Well No. |
| | | 11 | 12 | | | | | *44:25, 44:95 |
| | | | 12 | | | | | *44:50, 57:45 |
| | | | | | | | | *45:01, 45:03, 45:05-45:07, 45:11-45:13 |
| | | | | | | | | *45:04, 45:08 |
| 9 | | | | | | | | *45:10 |
| | | | | | | | | *50:01:01-50:01:03, 50:04-50:08, 50:10-50:11, 50:15 |
| | | | | | | | | *50:02 |
| | | | 12 | | | | | *50:13 |
| | | | | | | | | *51:07:01, 52:01:02, 52:01:04, 52:01:09, 52:02-52:03, 52:09, 52:21 |
| | | 11 | | | | | | *51:08, 51:20, 51:36, 51:44N, 52:19 |
| | | | | | | | | *51:62 |
| | | 11 | | | | | | *51:97 |
| | | | | | | | | *51:106 |
| | | | | | | | | *51:112 |
| | | | | | | | | *52:25 |
| | | | | w | | | | *54:10, 55:37 |
| | | | | | | | | *54:16, 54:20, 55:21, 55:46, 55:52, 56:13 |
| | | | | | | | 16 | *55:01:04 |
| | | | | 13 | | | | *55:39, 55:42 |
| | | | | | | | | *55:44, 55:53 |
| | | | | | | | | HLA-B allele |
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Well No. |

[4]The B*49:08 and 49:16 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 10.
[5]The B*49:13 and 49:14 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 15.
[6]Primer mix 2: Specific PCR fragment of 145 bp in the B*49:06 and the B*58:13 alleles. Specific PCR fragment of 255 bp in the B*49:02 and in the B*13:23, 18:09, 27:01, 37:10, 40:47, 40:96, 40:157, 44:15, 44:55, 44:103 alleles.
Primer mix 4: Specific PCR fragment of 170 bp in the B*49:03 and in the B*18:04, 40:01:11, 40:58, 44:02:10, 44:34, 51:07:01, 52:01:02, 52:01:04, 52:01:09, 52:02-52:03, 52:09 and 52:21 alleles. Specific PCR fragment of 205 bp in the B*49:09 and in the B*13:15, 51:62, 51:106 and 52:25 alleles.
Primer mix 7: Specific PCR fragment of 105 bp in the B*49:04 and 49:05 and in the B*13:04, 13:46, 15:04, 15:16:01-15:16:03, 15:67, 15:95, 15:137, 15:155, 15:222, 35:37, 39:06:01-39:06:02, 39:33-39:34, 39:50, 39:57, 39:62, 39:64, 40:86, 40:159, 45:02, 45:09, 50:09, 51:13:01-51:13:02,

51:37, 51:63, 51:92, 51:97, 52:14, 54:14, 55:13, 55:23, 55:32, 56:22, 59:03 and 73:01-73:02 alleles. Specific PCR fragment of 255 bp in the B*49:20 and in the B*44:15, 44:18, 44:20, 44:47, 44:100, 45:01-45:03, 45:05-45:07, 45:09, 45:11-45:13, 50:02, 51:23, 51:108 and 52:15 alleles.

Primer mix 8: Specific PCR fragment of 70 bp in the B*49:05 and in the B*07:78, 07:84, 08:09, 13:02:01-13:02:05, 13:02:07-13:04, 13:08Q-13:09, 13:14-13:16, 13:18-13:19, 13:27, 13:30-13:35, 13:37-13:38, 13:40-13:42, 13:44-13:49N, 15:04, 15:16:01-15:16:02, 15:42, 15:67, 15:83, 15:95, 15:137, 15:155, 15:222, 27:14, 27:81, 35:60, 39:06:01-39:06:02, 39:33-39:34, 39:50, 39:57, 39:62, 39:64, 40:06:01:01-40:06:04, 40:44, 40:53, 40:70, 40:75, 40:83, 40:86, 40:93, 40:95-40:96, 40:103, 40:109-40:110, 40:127, 40:131, 40:148, 40:159, 40:161-40:162, 40:165, 40:177, 41:01, 41:05-41:07, 41:09, 41:12, 41:14, 41:16-41:17, 42:04, 44:20, 44:47, 44:100, 46:11, 46:18, 51:01:01-51:01:18, 51:01:20-51:03, 51:05, 51:07:01-51:16, 51:18-51:24:04, 51:26-51:41N, 51:43-51:44N, 51:48-51:55, 51:57-51:58, 51:60-51:61, 51:63, 51:65-51:80, 51:82-51:93, 51:95-51:126, 52:01:01:01-52:27, 54:01:01-54:05N, 54:07-54:08N, 54:10-54:14, 54:16-54:24, 55:01:01-55:01:06, 55:01:08-55:03, 55:05, 55:07, 55:09-55:13, 55:15-55:26, 55:28-55:48, 55:50, 55:52-55:54, 56:01:01-56:01:04, 56:05:01-56:08, 56:13-56:17, 56:19N-56:30, 58:08:01-58:08:02, 59:01:01:01-59:05, 73:01-73:02 and 78:01:01-78:07 alleles. Specific PCR fragment of 155 bp in the B*49:19N allele.

Primer mix 10: Specific PCR fragment of 80 bp in the B*49:16 allele. Specific PCR fragment of 250 bp in the B*49:08 allele.

Primer mix 14: Specific PCR fragment of 155 bp in the B*49:19N allele. Specific PCR fragment of 190 bp in the B*49:12 and 49:17 alleles.

Primer mix 15: Specific PCR fragment of 160 bp in the B*49:13 allele. Specific PCR fragment of 190 bp in the B*49:17 allele. Specific PCR fragment of 290 bp in the B*49:14 allele.

Primer mix 16: Specific PCR fragment of 95 bp in the B*49:18 and in the B*35:01:10, 35:04:02, 40:28, 50:14, 51:56:01-51:56:02 and 55:01:04 alleles. Specific PCR fragment of 135 bp in the B*49:15 allele.

'w', may be weakly amplified.

# CELL LINE VALIDATION SHEET

## HLA-B*49 SSP primer set

| | | | | | Well | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| | | | | Prod. No.: | 201191101 | 201191102 | 201191103 | 201191104 | 201191105 | 201191106 | 201191107 | 201191108 | 201191109 | 201191110 | 201191111 | 201191112 | 201191113 | 201191114 | 201191115 | 201191116 |
| | IHWC cell line | | B* | | | | | | | | | | | | | | | | | |
| 1 | 9001 | SA | *07:02 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 9280 | LK707 | *52:01 | *73:01 | - | - | - | - | - | - | + | + | + | - | - | - | - | - | - | - |
| 3 | 9011 | E4181324 | *52:01 | | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| 4 | 9275 | GU373 | *15:10 | *53:01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 9009 | KAS011 | *37:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 9353 | SM | *39:01 | *51:01 | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| 7 | 9020 | QBL | *18:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 8 | 9025 | DEU | *35:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 9 | 9026 | YAR | *38:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 10 | 9107 | LKT3 | *54:01 | | - | - | - | - | + | + | - | + | - | - | - | - | + | - | - | - |
| 11 | 9051 | PITOUT | *44:03 | | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - | - |
| 12 | 9052 | DBB | *57:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 13 | 9025 | JESTHOM | *27:05 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 14 | 9071 | OLGA | *15:01 | *15:20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 15 | 9075 | DKB | *40:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 16 | 9037 | SWEIG007 | *40:02 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 17 | 9282 | CTM3953540 | *08:01 | *55:01 | - | - | - | - | + | + | - | + | - | - | - | - | - | - | - | - |
| 18 | 9257 | 32367 | *14:01 | *56:01 | - | - | - | - | + | + | - | + | - | - | - | - | + | - | - | - |
| 19 | 9038 | BM16 | *18:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 20 | 9059 | SLE005 | *40:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 21 | 9064 | AMALA | *15:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 22 | 9056 | KOSE | *35:03 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 23 | 9124 | IHL | *40:02 | *56:02 | - | - | - | - | - | + | - | - | - | - | - | - | + | - | - | - |
| 24 | 9035 | JBUSH | *38:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 25 | 9049 | IBW9 | *14:02 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 26 | 9285 | WT49 | *58:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 27 | 9191 | CH1007 | *07:05 | *51:01 | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| 28 | 9320 | BEL5GB | *44:02 | *44:03 | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - | - |
| 29 | 9050 | MOU | *44:03 | | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - | - |
| 30 | 9021 | RSH | *42:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 31 | 9019 | DUCAF | *18:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 32 | 9297 | HAG | *41:02 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 33 | 9098 | MT14B | *40:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 34 | 9104 | DHIF | *38:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 35 | 9302 | SSTO | *44:02 | | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - | - |
| 36 | 9024 | KT17 | *15:01 | *35:01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 37 | 9065 | HHKB | *07:02 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 38 | 9099 | LZL | *15:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 39 | 9315 | CML | *08:01 | *27:05 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 40 | 9134 | WHONP199 | *13:02 | *46:01 | - | - | - | - | + | + | - | + | + | - | - | - | + | - | - | - |
| 41 | 9055 | H0301 | *14:02 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 42 | 9066 | TAB089 | *46:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 43 | 9076 | T7526 | *46:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 44 | 9057 | TEM | *38:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 45 | 9239 | SHJO | *42:01 | *50:01 | - | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - |
| 46 | 9013 | SCHU | *07:02 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 47 | 9045 | TUBO | *51:01 | | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| 48 | 9303 | TER-ND | *35:01 | *44:03 | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - | - |

# CERTIFICATE OF ANALYSIS

## *Olerup* SSP® HLA-B*49 SSP

**Product number:** **101.547-06 – including *Taq* polymerase**
**Lot number:** **74M**
**Expiry date:** **2014-June-01**
**Number of tests:** **6**
**Number of wells per test:** **16**

**Well specifications:**

| Well No. | Production No. | Well No. | Production No. |
|---|---|---|---|
| 1 | 2011-911-01 | 9 | 2011-911-09 |
| 2 | 2011-911-02 | 10 | 2011-911-10 |
| 3 | 2011-911-03 | 11 | 2011-911-11 |
| 4 | 2011-911-04 | 12 | 2011-911-12 |
| 5 | 2011-911-05 | 13 | 2011-911-13 |
| 6 | 2011-911-06 | 14 | 2011-911-14 |
| 7 | 2011-911-07 | 15 | 2011-911-15 |
| 8 | 2011-911-08 | 16 | 2011-911-16 |

The specificity of each primer solution of the HLA-B*49 primer set has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 2, 4, 10, 11 and 14 to 16 were available. The specificities of the primers in primer solutions 2, 4, 11 and 16 were tested by separately adding additional 3'-primers, respectively additional 5'-primers. In primer solution 10, 14 and 15, it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 2 and 16, one 5'-primer was not possible to test, and in primer solution 8, one 3'-primer was not possible to test.

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

***Date of approval:*** 2011-December-15

***Approved by:***

**Production Quality Control**

# Declaration of Conformity

**Product name:** *Olerup* SSP® HLA-B*49
**Product number:** 101.547-06
**Lot number:** 74M

**Intended use:** HLA-B*49 high resolution histocompatibility testing

**Manufacturer:** *Olerup* SSP AB
Franzengatan 5
SE-112 51 Stockholm, Sweden
***Phone:*** +46-8-717 88 27
***Fax:*** +46-8-717 88 18

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 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, Franzengatan 5, SE-112 51 Stockholm, Sweden.

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

Stockholm, Sweden
2011-December-15

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

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***Web page:*** http://www.olerup.com

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