

Olerup SSP[®]

Olerup SSP[®] DQB1*06

Prod. No: 101.212-24/24u
101.212-04/04u

Lot No: 02G

Expiry Date: 2011-June-01

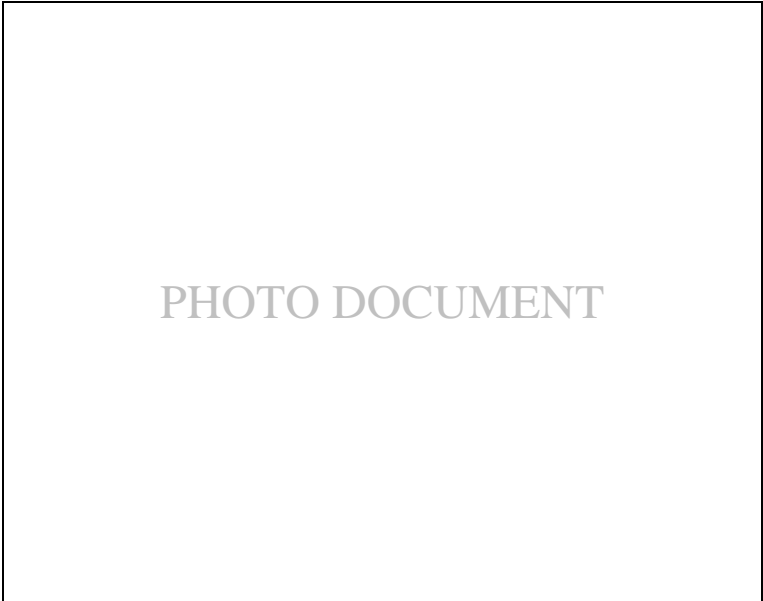
Name: _____ **Sample ID:** _____

DNA Extract Date: _____ **Conc.(ng/ul):** _____

Test Date: _____ **Review Date:** _____

Tested By: _____ **Reviewed By:** _____

Interpretation: _____



Specificities and sizes of the PCR products of the 29 primer mixes used for DQB1*06 SSP typing.

| Primer Mix | Size of spec. PCR product ¹ | Size of control band ² | Amplified DQB1 alleles ³ |
|-------------------|--|-----------------------------------|---|
| 1 | 220 bp | 515 bp | 060101-060104 |
| 2 | 210 bp | 430 bp | 060101-060202, 060502 [?] , 0606 [?] , 0610-061102, 0613, 0616, 0618-0620, 0624, 0629, 0633 |
| 3 | 185 bp | 430 bp | 060201-060202, 061401-0616, 0619, 0620, 0623, 0624, 0633 |
| 4 | 130 bp | 430 bp | 060301-060302, 0607, 061102, 061401, 0626N, 0628, 0630-0632 |
| 5 | 160 bp | 430 bp | 060301-060302, 060402, 0607, 060801, 061101-061102, 0626N, 0628, 0630-0632, 0323 |
| 6 | 170 bp | 515 bp | 060301-060302, 060801-060802, 061102, 0612, 061401, 0621, 0626N, 0628, 0631 |
| 7 | 210 bp | 515 bp | 060401-060403, 0607, 0617, 0621, 0625, 0634 |
| 8 | 170 bp | 430 bp | 060401-060501, 060502, 0606, 0607, 0609, 0618, 0625, 0627, 0632, 0634 |
| 9 ⁵ | 130 bp | 430 bp | 060401-060501, 0606, 060801-0609, 0612, 0617, 0618, 0621, 0627, 0634 |
| 10 ⁶ | 260 bp | 515 bp | 060501, 060502 [?] , 0606 [?] , 0620, 0631 |
| 11 | 210 bp | 430 bp | 060501, 060502 [?] , 0606 [?] , 0609, 0612, 0615 ^w , 0622 ^w |
| 12 | 180 bp | 430 bp | 0606 |
| 13 | 185 bp | 430 bp | 0610 |
| 14 | 130 bp | 430 bp | 0613, 0622 |
| 15 ^{4,8} | 100, 185 bp | 430 bp | 061401-061402, 0629 |
| 16 | 195 bp | 430 bp | 0616 |
| 17 ⁴ | 110 bp | 430 bp | 0623 |
| 18 ⁷ | 175 bp | 430 bp | 0617, 0624, 0630 |
| 19 | 135 bp | 430 bp | 0610, 0625 |
| 20 | 215 bp | 515 bp | 0626N |
| 21 | 160 bp | 430 bp | 060201-060202, 0610, 0613-0616, 0620, 0623, 0624, 0629, 0633 |
| 22 | 130 bp | 515 bp | 0607, 0615 |
| 23 | 160 bp | 515 bp | 060301-060302, 060801-060802, 061401-061402, 0621, 0628, 0631 |
| 24 | 155 bp | 430 bp | 0619, 0401- 0403 |
| 25 | 210 bp | 430 bp | 060301-060302, 060801-060802, 061401-061402, 0627, 0628, 0630-0632 |
| 26 | 190 bp | 430 bp | 0628 |
| 27 | 265 bp | 430 bp | 0633 |
| 28 | 300 bp | 430 bp | 0634 |
| 29 ⁴ | 90 bp | 430 bp | 060401-060501, 060502 [?] , 0606 [?] , 0607, 0609, 0615, 0622, 0625, 0634 |

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 DQB1*06 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

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DQB1*06 subtyping.

In addition, wells number 6, 7, 10, 20, 22 and 23 contain the primer pair giving rise to the longer, 515 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.

³Due to the sharing of sequence motif between DQB1 alleles, the DQB1*0323 alleles will be amplified by primer mix 5, and the DQB1*0401 to 0403 alleles will be amplified by primer mix 24.

⁴Specific PCR fragments shorter than 125 base pairs have a lower intensity than longer PCR bands.

⁵Primer mix 9 may yield somewhat less intense specific PCR fragments than the other DQB1*06 primer mixes.

⁶The nucleotide sequence of codon 14 of the DQB1*060502 allele is not yet known. If codon 14 is CTg, then the DQB1*060502 allele will retain its name and will be amplified by the primer pair in well No. 10. If the sequence of codon 14 is ATg, then DQB1*060502 will be renamed to DQB1*060902 (Steven Marsh personal communication), and will not be amplified by the primer pair in well No. 10.

⁷Primer mix 18 may give rise to primer dimer formation.

⁸Primer mix 15: Specific PCR fragment of 100 bp in the DQB1*061401 and *061402 alleles. Specific PCR fragment of 185 bp in the DQB1*0629 allele.

“?”, nucleotide sequence information is not available for the primer matching sequence.

‘w’, might be weakly amplified.

| Well No. | 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 |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| DQB1 allele | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *060101-060104 | 1 | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *060201-060202 | | 2 | 3 | | | | | | | | | | | | | | | | | | 21 | | | | | | | | |
| *060301-060302 | | | | 4 | 5 | 6 | | | | | | | | | | | | | | | | | 23 | | 25 | | | | |
| *060401,060403 | | | | | | | 7 | 8 | 9 | | | | | | | | | | | | | | | | | | | | 29 |
| *060402 | | | | | 5 | | 7 | 8 | 9 | | | | | | | | | | | | | | | | | | | | 29 |
| *060501 | | | | | | | | 8 | 9 | 10 | 11 | | | | | | | | | | | | | | | | | | 29 |
| *060502 | | ? | | | | | | 8 | | ? | ? | | | | | | | | | | | | | | | | | | ? |
| *0606 | | ? | | | | | | 8 | 9 | ? | ? | 12 | | | | | | | | | | | | | | | | | ? |
| *0607 | | | | 4 | 5 | | 7 | 8 | | | | | | | | | | | | | | | 22 | | | | | | 29 |
| *060801 | | | | | 5 | 6 | | | 9 | | | | | | | | | | | | | | 23 | | 25 | | | | |
| *060802 | | | | | 6 | | | | 9 | | | | | | | | | | | | | | 23 | | 25 | | | | |
| *0609 | | | | | | | | 8 | 9 | | 11 | | | | | | | | | | | | | | | | | | 29 |
| *0610 | | 2 | | | | | | | | | | | 13 | | | | | | 19 | | 21 | | | | | | | | |
| *061101 | | 2 | | | 5 | | | | | | | | | | | | | | | | | | | | | | | | |
| *061102 | | 2 | | 4 | 5 | 6 | | | | | | | | | | | | | | | | | | | | | | | |
| *0612 | | | | | 6 | | | | 9 | | 11 | | | | | | | | | | | | | | | | | | |
| *0613 | | 2 | | | | | | | | | | | 14 | | | | | | | | 21 | | | | | | | | |
| *061401 | | | 3 | 4 | | 6 | | | | | | | | 15 | | | | | | | 21 | 23 | | | 25 | | | | |
| *061402 | | | 3 | | | | | | | | | | | 15 | | | | | | | 21 | 23 | | | 25 | | | | |
| *0615 | | | 3 | | | | | | | | w | | | | | | | | | | 21 | 22 | | | | | | | 29 |
| *0616 | | 2 | 3 | | | | | | | | | | | | 16 | | | | | | 21 | | | | | | | | |
| *0617 | | | | | | 7 | | | 9 | | | | | | | | | 18 | | | | | | | | | | | |
| *0618 | | 2 | | | | | 8 | | 9 | | | | | | | | | | | | | | | | | | | | |
| *0619 | | 2 | 3 | | | | | | | | | | | | | | | | | | | | 24 | | | | | | |
| *0620 | | 2 | 3 | | | | | | | 10 | | | | | | | | | | | 21 | | | | | | | | |
| *0621 | | | | | 6 | 7 | | | 9 | | | | | | | | | | | | | | 23 | | | | | | |
| *0622 | | | | | | | | | | | w | | 14 | | | | | | | | | | | | | | | | 29 |
| *0623 | | | 3 | | | | | | | | | | | | | | | 17 | | | 21 | | | | | | | | |
| *0624 | | 2 | 3 | | | | | | | | | | | | | | | 18 | | | 21 | | | | | | | | |
| *0625 | | | | | | | 7 | 8 | | | | | | | | | | | 19 | | | | | | | | | | 29 |
| *0626N | | | | 4 | 5 | 6 | | | | | | | | | | | | | | 20 | | | | | | | | | |
| *0627 | | | | | | | | 8 | 9 | | | | | | | | | | | | | | | | | | 25 | | |
| *0628 | | | | 4 | 5 | 6 | | | | | | | | | | | | | | | | | 23 | | 25 | 26 | | | |
| *0629 | | 2 | | | | | | | | | | | | 15 | | | | | | | 21 | | | | | | | | |
| *0630 | | | | 4 | 5 | | | | | | | | | | | | | | 18 | | | | | | | | 25 | | |
| *0631 | | | | 4 | 5 | 6 | | | | 10 | | | | | | | | | | | | | 23 | | 25 | | | | |
| *0632 | | | | 4 | 5 | | | 8 | | | | | | | | | | | | | | | | | | | 25 | | |
| *0633 | | 2 | 3 | | | | | | | | | | | | | | | | | | 21 | | | | | | | 27 | |
| *0634 | | | | | | | 7 | 8 | 9 | | | | | | | | | | | | | | | | | | | 28 | 29 |
| *0323 | | | | | 5 | | | | | | | | | | | | | | | | | | | | | | | | |
| *0401-0403 | | | | | | | | | | | | | | | | | | | | | | | | 24 | | | | | |
| Length of spec. PCR product(s)¹ | 220 | 210 | 185 | 130 | 160 | 170 | 210 | 170 | 130 | 260 | 210 | 180 | 185 | 130 | 100 | 195 | 110 | 175 | 135 | 215 | 160 | 130 | 160 | 155 | 210 | 190 | 265 | 300 | 90 |
| Well No. | 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 |
| Specific Reaction | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1st match: DQB1* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2nd match: DQB1* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Control Reaction | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Failed Controls | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| False Positive | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| False Negative | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

¹Primer mix 15: Specific PCR fragment of 100 bp in the DQB1*061401 and *061402 alleles. Specific PCR fragment of 185 bp in the DQB1*0629 allele.

"?", nucleotide sequence information is not available for the primer matching sequence.

'w', might be weakly amplified.

Change in revision R01 compared to R00:

- The DQB1*0615 and DQB1*0622 alleles are weakly amplified by primer mix 11. This has been changed in the Specificity and Interpretation Tables.