Gel Documentation Form and Worksheet

DQA1\*02,05;DQB1\*02,03:02 Lot No: 3E2 Expiry Date: 2019-04-01

(101.903-24/24u)

Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sample ID:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

DNA Conc.(ng/ul):\_\_\_\_\_\_\_\_\_

Test Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Tested By:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Review Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Reviewed By:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*Interpretation:\_\_\_\_\_\_\_\_\_\_\_ Failed lanes*: \_\_\_\_\_\_\_\_\_\_\_\_ *Comments:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

**Gel Picture**

|  |
| --- |
| PHOTO DOCUMENT |



‘ICB’ Internal Control Band,

‘AmpS’ Amplicon size

**Notes:**

Product sizes are approximate. For detailed information, see the lot-specific Specificity Table and Interpretation Table.

This table is intended as a guide. For interpretation always use the Interpretation Table and/or Specificity Table.

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

The primer pair in well 17 will in some samples give rise to two HLA-specific PCR fragments and may give rise to a lower yield for the DQB1\*03xx alleles.

Primer mix 12 may have tendencies of unspecific amplification.

Primer mix 7 may give rise to a lower yield of HLA-specific PCR product than the other DQA1\*02,05;DQB1\*02,03:02 primer mixes.

In primer mix 18 the positive control band may be weaker than for other DQA1\*02,05;DQB1\*02,03:02 primer mixes.

Primer mix 21 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.





 **1**DQA1 and DQB1 alleles listed on the IMGT/HLA web page 2016-July-14, release 3.25.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

Bold lettering alleles are DQA1\*02,05;DQB1\*02,03:02 associated alleles.

**2**Alleles 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 <http://hla.alleles.org/alleles/deleted.html>.

**3**Primer mix 4: Specific PCR fragment of 90 bp in the DQA1\*05:02 allele. Specific PCR fragment of 200 bp in the DQB1\*05:02?, 05:03, 05:04? and 05:06-05:07 alleles.

Primer mix 9: Specific PCR fragment of 145 bp in the DQA1\*01:03:01:01-01:03:01:06 and 01:10 alleles. Specific PCR fragment of 170 bp in the DQA1\*01:01:01:01-01:01:03, 01:04:01:01-01:05:02, 01:07Q and 01:12 alleles. Specific PCR fragment of 145 bp and 170 bp in the DQA1\*01:02:01:01-01:02:04, 01:06, 01:08-01:09, 01:11 and 01:13 alleles.

Primer mix 16: Specific PCR fragment of 135 bp in the DQB1\*03:04:01-03:04:02, 03:09, 03:14:01-03:14:02, 03:80 and 03:138 alleles. Specific PCR fragment of 170 bp in the DQB1\*03:11 allele.

Primer mix 17: Specific PCR fragment of 145 bp in the DQB1\*03:01:02, 03:02:03, 03:02:13, 03:03:03, 03:05:01-03:05:02, 03:17:02, 03:20, 03:23:01, 03:37, 03:61, 03:72-03:73, 03:75, 03:99Q-03:100, 03:112, 03:118N, 03:132, 03:156, 03:162, 03:181, 03:183, 03:204, 03:226, and 04:09 alleles.

Specific PCR fragment of 185 bp in the DQB1\*03:23:02, 03:25:01-03:25:02, 03:153, 03:172, 03:205, 03:214, 03:217 and 04:01:03 alleles. Specific PCR fragment of 145 and 185 bp in the DQB1\*03:01:01:01-03:01:01:03, 03:01:03-03:02:02, 03:02:04-03:02:12, 03:02:14-03:03:02:04, 03:03:04-03:04:02, 03:05:03-03:17:01, 03:18-03:19:02, 03:21-03:22, 03:24, 03:26-03:36, 03:38-03:60, 03:62-03:71, 03:74, 03:76-03:98, 03:101-03:103, 03:106-03:108, 03:110-03:111, 03:113-03:117, 03:119-03:131, 03:133-03:152, 03:155, 03:157-03:161, 03:163-03:171, 03:173-03:180, 03:182, 03:184-03:188, 03:190-03:203, 03:206-03:213N, 03:215-03:216, 03:218-03:222, 03:224-03:225, 03:227-03:230 alleles. Both bands may not always be visible.

The DQA1\*02,05;DQB1\*02,03:02 subtyping kit cannot distinguish the silent mutations in the DQA1\*03:01:01 and 03:01:03, the DQA1\*03:03:01-03:03:02, the DQA1\*05:01:01:01-05:01:02 and DQA1\*05:05:01:01-05:05:01:06 alleles, the DQB1\*02:01:01-02:01:06 and 02:01:08-02:01:20, 02:01:22-02:01:24 the DQB1\*02:01:07 and 02:01:21, the DQB1\*02:02:01:01-02:02:02, the DQB1\*03:01:01:01-03:01:07 and 03:01:09-03:03:33, the DQB1\*03:02:01-03:02:09, 03:02:12-03:02:13 and 03:02:15-03:02:19 or the DQB1\*03:02:10 and 03:02:14 alleles.

‘?’, nucleotide sequence information not available for the primer matching sequence.

Expected Results

Table 1 describes expected results for the (groups of) alleles that the kit is able to detect and separate.

Table 1: Expected results for targeted DQA1 and DQB1 alleles.

|  |  |  |  |
| --- | --- | --- | --- |
| **DQA1 alleles** | **DQB1 alleles** | **Positive DQA1 wells** | **Positive DQB1 wells** |
| 05:01 | 02:01 (DQ2) | 3, 5 | 10, 12, 14 |
|   |  |  |  |
| *(02:01)*05:05 | 02:02 (DQ2)*(03:01)* | 1, 83, 5, 6 | 10, 11, 1412, 17, 19 |
|  |  |  |  |
| 03:01 | 03:02 (DQ8) | 2, 8 | 12, 14, 15, 17, 20 |
| 03:02, 03:03 | 03:02 (DQ8) | 2 | 12, 14, 15, 17, 20 |
|  |  |  |  |
| 05:01 |   | 3, 5 |  |
| 05:05 |   | 3, 5, 6 |  |
| 02:01 |   | 1, 8 |  |
| 03 |   | 2, 8 |  |
|  |  |  |  |
|   | 02:01 |  | 10, 12, 14 |
|   | 02:02 |  | 10, 11, 14 |
|   | 03:01 |  | 12, 17, 19 |
|  | 03:02 |  | 12, 14, 15, 17, 20 |

The negative control DNA must only give rise to the internal control bands of 430 or 515 base pairs respectively and no DQA1\*02,05;DQB1\*02,03:02specific bands. Additional bands might indicate inappropriate test conditions or contamination.