Gel Documentation Form and Worksheet

DQB1\*06

(101.212-24/04 – 24u/04u) Lot No: 5E8 Expiry Date: 2019-06-01

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.

Primer mixes 9, 16, 24 and 32 may give rise to a lower yield of HLA-specific PCR product than the other DQB1\*06 primer mixes.

Primer mixes 19, 20, 32, 40 and 51 have a tendency to giving rise to primer oligomer formation.

Primer mixes 8, 9, 10, 15, 20, 31, 34, 38, 41, 51, 57 and 62 may have tendencies of unspecific amplifications.

In primer mix 32 the positive control band may be weaker than for other DQB1\*06 primer mixes.

The nucleotide sequence of codon 14 of the DQB1\*06:05:02 allele is not yet known. If codon 14 is CTg, then the DQB1\*06:05:02 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\*06:05:02 will be renamed to DQB1\*06:09:02 (Steven Marsh personal communication), and will not be amplified by the primer pair in well No. 10.

Primer mix 64 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**HLA-DQB1 in bold lettering are listed as confirmed alleles 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).

**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 8: Specific PCR fragment of 90 bp in the DQB1\*06:65 allele. Specific PCR fragment of 175 bp in the DQB1\*06:04:01-06:07:02, 06:09:01-06:09:03, 06:09:05-06:09:06, 06:18:01-06:18:02, 06:22:02, 06:25, 06:27:01-06:27:02, 06:32:01-06:32:02, 06:34, 06:36, 06:38-06:39, 06:52, 06:58, 06:66, 06:69, 06:85-06:86, 06:88-06:89, 06:93-06:94, 06:118:01-06:118:02, 06:121, 06:129, 06:135, 06:142, 06:149, 06:155, 06:158N, 06:160, 06:164, 06:168, 06:171-06:172, 06:180, 06:186, 06:189, 06:193N, 06:202, 06:204 and 06:207 alleles.

Primer mix 11: Specific PCR fragment of 155 bp in the DQB1\*06:164 allele. Specific PCR fragment of 210 bp in the DQB1\*06:05:01, 06:05:02?-06:06?, 06:09:01-06:09:06, 06:12, 06:15:01-06:15:02, 06:22:01-06:22:03, 06:42, 06:46, 06:66, 06:88, 06:94, 06:118:01-06:119, 06:121, 06:142, 06:189 and 06:207 alleles.

Primer mix 12: Specific PCR fragment of 100 bp in the DQB1\*06:64 allele. Specific PCR fragment of 135 bp in the DQB1\*06:94 and 06:170 alleles. Specific PCR fragment of 180 bp in the DQB1\*06:06 and 06:149 alleles. Specific PCR fragment of 215 bp in the DQB1\*06:06? and 06:129 alleles.

Primer mix 13: Specific PCR fragment of 185 bp in the DQB1\*06:10 and 06:130 alleles. Specific PCR fragment of 225 bp in the DQB1\*06:05:02?, 06:15:01-06:15:02, 06:22:01-06:22:03, 06:37, 06:48, 06:51:01-06:51:02, 06:69 and 06:139 and the DQB1\*03:30, 03:72, 03:100 and 03:215 alleles.

Primer mix 14: Specific PCR fragment of 130 bp in the DQB1\*06:09:04, 06:13:01-06:13:02, 06:22:01, 06:22:03, 06:55, 06:119 and 06:206 alleles. Specific PCR fragment of 215 bp in the DQB1\*06:146:01-06:146:02 alleles.

Primer mix 15: Specific PCR fragment of 105 bp in the DQB1\*06:14:01-06:14:03, 06:69, 06:156, 06:162 and 06:206 and the DQB1\*04:28, 05:38, 05:62 and 05:119 alleles. Specific PCR fragment of 185 bp in the DQB1\*06:29, 06:123 and 06:139 and the DQB1\*03:132 and 03:215 alleles.

Primer mix 16: Specific PCR fragment of 195 bp in the DQB1\*06:16 allele. Specific PCR fragment of 215 bp in the DQB1\*06:51:01-06:51:02, 04:01:01w-04:03:02w and 04:06w-04:36Nw alleles.

Primer mix 18: Specific PCR fragment of 145 bp in the DQB1\*06:193N and the DQB1\*03:66N alleles. Specific PCR fragment of 185 bp in the DQB1\*06:17, 06:24, 06:30, 06:42 and 06:149 and the DQB1\*03:228 alleles. Specific PCR fragment of 225 bp in the DQB1\*06:171 allele.

Primer mix 19: Specific PCR fragment of 140 bp in the DQB1\*06:10, 06:25, 06:36, 06:130 and 06:193N and the DQB1\*03:66N alleles. Specific PCR fragment of 225 bp in the DQB1\*06:171 allele.

Primer mix 20: Specific PCR fragment of 110 bp in the DQB1\*06:37 and 06:125 alleles. Specific PCR fragment of 210 bp in the DQB1\*06:26N and 06:81 alleles. Specific PCR fragment of 260 bp in the DQB1\*06:83 allele.

Primer mix 22: Specific PCR fragment of 130 bp in the DQB1\*06:07:01-06:07:02, 06:15:01-06:15:02, 06:46, 06:66, 06:92, 06:118:01-06:118:02 and 06:172 alleles. Specific PCR fragment of 195 bp in the DQB1\*06:38 and 06:158N alleles.

Primer mix 25: Specific PCR fragment of 210 bp in the DQB1\*06:03:01-06:03:22, 06:08:01-06:08:03, 06:14:01-06:14:03, 06:27:01-06:28, 06:30-06:32:02, 06:40-06:41, 06:44, 06:59-06:65, 06:67, 06:87, 06:90-06:91, 06:98, 06:110, 06:128, 06:133-06:134, 06:141, 06:143-06:145, 06:148, 06:154, 06:168, 06:170, 06:184-06:185, 06:187, 06:190-06:191, 06:195-06:196 and 06:206 alleles. Specific PCR fragment of 260 bp in the DQB1\*06:05:02?, 06:06? and 06:49 alleles.

Primer mix 26: Specific PCR fragment of 165 bp in the DQB1\*06:35, 06:53:01-06:53:02, 06:145 and 06:208 alleles. Specific PCR fragment of 190 bp in the DQB1\*06:28, 06:56 and 06:79:01-06:79:02 and the DQB1\*05:73, 05:98 and 05:116 alleles.

Primer mix 27: Specific PCR fragment of 155 bp in the DQB1\*06:114 allele. Specific PCR fragment of 195 bp in the DQB1\*06:40, 06:81 and 06:132 alleles.

Specific PCR fragment of 220 bp in the DQB1\*06:57 alleles. Specific PCR fragment of 265 bp in the DQB1\*06:33 allele.

Primer mix 28: Specific PCR fragment of 130 bp in the DQB1\*06:102N allele. Specific PCR fragment of 180 bp in the DQB1\*06:50 allele. Specific PCR fragment of 300 bp in the DQB1\*06:34 allele.

Primer mix 31: Specific PCR fragment of 100 bp in the DQB1\*06:44 and 06:47 alleles. Specific PCR fragment of 220 bp in the DQB1\*06:43 allele.

Primer mix 35: Specific fragment of 135 bp in the DQB1\*06:66 and 06:172 alleles. Specific PCR fragment of 185 bp in the DQB1\*06:54N and 06:135 alleles. Specific PCR fragment of 260 bp in the DQB1\*06:05:02?, 06:06? and 06:58 alleles.

Primer mix 37: Specific PCR fragment of 120 bp in the DQB1\*06:80 allele. Specific PCR fragment of 175 bp in the DQB1\*06:29, 06:76-06:77N, 06:96 and 06:139 and the DQB1\*03:30, 03:72, 03:100, 03:132, 03:215 and 04:09 alleles. Specific PCR fragment of 245 bp in the DQB1\*06:05:02? and 06:137 alleles.

Primer mix 38: Specific PCR fragment of 170 bp in the DQB1\*06:78 and 06:123 alleles. Specific PCR fragment of 245 bp in the DQB1\*06:137 allele. Specific PCR fragment of 285 bp in the DQB1\*06:72-06:73 alleles.

Primer mix 39: Specific PCR fragment of 120 bp in the DQB1\*06:80 allele. Specific PCR fragment of 155 bp in the DQB1\*06:138 allele. Specific PCR fragment of 270 bp in the DQB1\*06:73-06:74 alleles.

Primer mix 40: Specific PCR fragment of 105 bp in the DQB1\*06:70 allele. Specific PCR fragment of 190 bp in the DQB1\*06:75N, 06:106 and 06:136 alleles.

Primer mix 42: Specific PCR fragment of 130 bp in the DQB1\*06:93-06:94 and 06:170 alleles. Specific PCR fragment of 165 bp in the DQB1\*06:121, 06:142 and 06:168 alleles. Specific PCR fragment of 190 bp in the DQB1\*06:60-06:61 alleles.

Primer mix 43: Specific PCR fragment of 150 bp in the DQB1\*06:103 and the DQB1\*05:14 and 05:84 alleles. Specific PCR fragment of 180 bp in the DQB1\*06:07:01?, 06:20?, 06:68 and 06:131 and the DQB1\*05:03:02? alleles.

Primer mix 44: Specific PCR fragment of 130 bp in the DQB1\*06:113 allele. Specific PCR fragment of 180 bp in the DQB1\*06:67, 06:174 and 06:191 alleles. Specific PCR fragment of 220 bp in the DQB1\*06:143 allele.

Primer mix 45: Specific PCR fragment of 150 bp in the DQB1\*06:97 allele. Specific PCR fragment of 195 bp in the DQB1\*06:136 allele. Specific PCR fragment of 235 bp in the DQB1\*06:124 allele.

Primer mix 46: Specific PCR fragment of 170 bp in the DQB1\*06:163 allele. Specific PCR fragment of 240 bp in the DQB1\*06:86, 06:104 and 06:107 and the DQB1\*03:97 alleles.

Primer mix 47: Specific PCR fragment of 95 bp in the DQB1\*06:29, 06:59, 06:63, 06:87, 06:96 and 06:150 and the DQB1\*03:08, 03:137 and 03:194 alleles. Specific PCR fragment of 180 bp in the DQB1\*06:90 allele. Specific PCR fragment of 220 bp in the DQB1\*06:143 allele.

Primer mix 48: Specific PCR fragment of 110 bp in the DQB1\*06:59, 06:91, 06:145, 06:150 and 06:208 and the DQB1\*03:194 alleles. Specific PCR fragment of 205 bp in the DQB1\*06:128 allele.

Primer mix 49: Specific PCR fragment of 190 bp in the DQB1\*06:100, 06:132 and 06:140 alleles. Specific PCR fragment of 230 bp in the DQB1\*06:126 allele.

Primer mix 50: Specific PCR fragment of 75 bp in the DQB1\*06:134 allele. Specific PCR fragment of 150 bp in the DQB1\*06:101 allele. Specific PCR fragment of 190 bp in the DQB1\*06:140 and 06:144N alleles. Specific PCR fragment of 275 bp in the DQB1\*06:120 allele.

Primer mix 51: Specific PCR fragment of 120 bp in the DQB1\*06:111 and 06:189 and the DQB1\*04:17, 05:31, 05:46 and 05:108 alleles. Specific PCR fragment of 240 bp in the DQB1\*06:205 allele.

Primer mix 52: Specific PCR fragment of 95 bp in the DQB1\*06:155 allele. Specific PCR fragment of 130 bp in the DQB1\*06:133 allele. Specific PCR fragment of 165 bp in the DQB1\*06:188 and 06:200 alleles. Specific PCR fragment of 195 bp in the DQB1\*06:105 and 06:185 and the DQB1\*05:47 alleles.

Primer mix 53: Specific PCR fragment of 150 bp in the DQB1\*06:109-06:110 and 06:200 and the DQB1\*03:115 alleles. Specific PCR fragment of 195 bp in the DQB1\*06:105 and 06:185 and the DQB1\*05:47 alleles. Specific PCR fragment of 220 bp in the DQB1\*06:147 allele.

Primer mix 56: Specific PCR fragment of 130 bp in the DQB1\*06:99:01-06:99:02 alleles. Specific PCR fragment of 200 bp in the DQB1\*06:116 and 06:187 alleles.

Primer mix 57: Specific PCR fragment of 125 bp in the DQB1\*06:07:01-06:07:02, 06:15:01-06:15:02, 06:46, 06:92 and 06:118:01-06:118:02 alleles. Specific PCR fragment of 185 bp in the DQB1\*06:89 and 06:135 and the DQB1\*05:80 alleles. Specific PCR fragment of 200 bp in the DQB1\*06:158N allele.

Primer mix 58: Specific PCR fragment of 215 bp in the DQB1\*06:153 and the DQB1\*03:52 and 03:179 alleles. Specific PCR fragment of 275 bp in the DQB1\*06:127 and 06:205 alleles. Specific PCR fragment of 300 bp in the DQB1\*06:52 allele.

Primer mix 59: Specific PCR fragment of 170 bp in the DQB1\*06:163 allele. Specific PCR fragment of 230 bp in the DQB1\*06:117 and 06:147 alleles.

Primer mix 60: Specific PCR fragment of 140 bp in the DQB1\*06:141 and 06:179N alleles. Specific PCR fragment of 180 bp in the DQB1\*06:191 allele.

**5**The following DQB1\*06 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |  |  |
| --- | --- | --- | --- |
| **Alleles** | **Primer mix** | **Alleles** | **Primer mix** |
| DQB1\*06:33, 06:114 | 27 | DQB1\*06:91, 06:128 | 48 |
| DQB1\*06:70, 06:75N | 40 | DQB1\*06:97, 06:124 | 45 |
| DQB1\*06:83, 06:125 | 20 | DQB1\*06:134, 06:144N | 50 |
|  |  |  |  |

The DQB1\*06 subtyping kit cannot distinguish the silent mutations in the DQB1\*06:01:01, 06:01:03-06:01:06, 06:01:08-06:01:11 and 06:01:13-06:01:15 alleles, the DQB1\*06:01:02, 06:01:07 and 06:01:12 alleles, the DQB1\*06:02:01-06:02:04, 06:02:06 and 06:02:08-06:02:25 alleles, the DQB1\*06:03:01-06:03:03, 06:03:05-06:03:06, 06:03:11-06:03:18 and 06:03:20-06:03:22 alleles, the DQB1\*06:03:04 and 06:03:08-06:03:10 alleles, the DQB1\*06:04:01 and 06:04:03-06:04:10 alleles, the DQB1\*06:08:02-06:08:03 alleles, the DQB1\*06:09:03 and 06:09:05-06:09:06 alleles, the DQB1\*06:11:02-06:11:03 alleles, the DQB1\*06:13:01-06:13:02 alleles, the DQB1\*06:14:01 and 06:14:03 alleles, the DQB1\*06:15:01-06:15:02 alleles, the 06:19:01-06:19:02 alleles, the DQB1\*06:22:01 and 06:22:03 alleles, the DQB1\*06:27:01-06:27:02 alleles, the DQB1\*06:79:01-06:79:02 alleles or the DQB1\*06:146:01-06:146:02 alleles.

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

‘w’, might be weakly amplified.

Change in revision R01 compared to R00:

1. In primer mixes 13, 21, 25 and 35 nucleotide sequence information is not available for the primer matching sequence in the DQB1\*06:05:02 allele. The amplification pattern has been changed to “?” in the Specificity and Interpretation Tables.