Gel Documentation Form and Worksheet

DRB1\*03 (101.113-24/04, -24u/04u) Lot No: 8E7 Expiry Date: 2019-09-01

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 6, 14, 22 and 23 may have a tendency of giving rise to primer oligomer formation.

Primer mixes 26 and 27 may have a tendency of unspecific amplification.

Primer mixes 2 and 24 may give rise to a lower yield of specific PCR product than the other DRB1\*03 primer mixes.

Primer mix 32 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**DRB1\*03 alleles in bold lettering are listed as confirmed alleles on the IMGT/HLA web page [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla), release 3.26.0, October 2016.

**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 3: Specific PCR fragment of 155 bp in the DRB1\*03:97 and 03:126 and the DRB1\*14:18, 14:33, 14:59, 14:64, 14:95, 14:106, 14:132-14:133 and 14:154 alleles. Specific PCR fragment of 190 bp in the DRB1\*03:02:01-03:02:03, 03:05:01-03:05:03, 03:09, 03:14, 03:27, 03:29, 03:38, 03:53, 03:74, 03:81, 03:88, 03:90, 03:102-03:103, 03:115, 03:122 and 03:130-03:131 and the DRB1\*11:09, 11:20, 11:28:02, 11:87, 11:122, 13:02:01-13:02:03, 13:02:05-13:02:12, 13:05:01-13:05:03, 13:26:01-13:26:02, 13:29, 13:31, 13:34, 13:36, 13:39, 13:41, 13:50:02-13:50:03, 13:56, 13:63, 13:65, 13:67, 13:73-13:74, 13:85, 13:96:01-13:96:02, 13:99, 13:103-13:104, 13:119-13:120, 13:123-13:124, 13:126, 13:128, 13:136, 13:139-13:140, 13:142N-13:143, 13:145, 13:147, 13:155, 13:157-13:159, 13:165, 13:168, 13:170-13:171:02, 13:179, 13:189, 13:196, 13:198-13:199, 13:202-13:203, 13:207-13:212, 13:220, 13:225, 14:02:01-14:03:02, 14:09, 14:13, 14:19, 14:24, 14:27:01-14:27:02, 14:30, 14:46-14:48, 14:63, 14:67, 14:85, 14:89, 14:98, 14:109, 14:115, 14:135, 14:144, 14:174, 14:179 and 16:08 and in the DRB3\*01:08, DRB3\*02:06 and 02:20 alleles. Specific PCR fragment of 155 bp and 190 bp in the DRB1\*14:51 and 14:134 alleles.

Primer mix 7: Specific PCR fragment of 125 bp in the DRB1\*03:08, 03:65 and 03:72 and the DRB1\*11:59, 11:80, 11:83, 11:87, 11:135, 11:142 and 11:182 alleles. Specific PCR fragment of 150 bp in the DRB1\*03:124 and in the DRB5\*01:04? alleles. Specific PCR fragment of 215 bp in the DRB1\*03:132 allele.

Primer mix 11: Specific PCR fragment of 100 bp in the DRB1\*03:09, 03:23 and 03:112 alleles. Specific PCR fragment of 125 bp in the DRB1\*03:115 and the DRB1\*14:151 alleles. Specific PCR fragment of 180 bp in the DRB1\*03:13:01-03:13:02, 03:38, 03:79 and 03:88 and the DRB1\*11:116, 11:120, 13:39, 13:54, 13:77-13:79, 13:100, 13:162-13:163, 13:181, 14:48, 14:64, 14:109, 14:115, 14:177 and in the DRB3\*01:14 and 01:25? alleles.

Primer mix 12: Specific PCR fragment of 135 bp in the DRB1\*03:10 and 03:110 and the DRB1\*14:82 and 14:132 alleles. Specific PCR fragment of 190 bp in the DRB1\*03:51, 03:76 and 03:125 and the DRB1\*11:59w, 11:80w, 11:83w, 11:87w, 11:135w, 11:142w, 11:182w, 13:27w, 13:41w, 13:71w, 13:129w, 13:176w and 14:179w alleles.

Primer mix 13: Specific PCR fragment of 125 bp in the DRB1\*03:115 and the DRB1\*14:151 alleles. Specific PCR fragment of 160 bp in the DRB1\*03:47 and 03:62 alleles. Specific PCR fragment of 225 bp in the DRB1\*03:11:01, 03:17, 03:24, 03:27, 03:35 and 03:81 and the DRB1\*11:136, 14:38:01-14:38:02 and 14:127:01-14:127:02 and in the DRB3\*02:54? alleles.

Primer mix 14: Specific PCR fragment of 105 bp in the DRB1\*03:16 allele. Specific PCR fragment of 135 bp in the DRB1\*03:12 and 03:110 and the DRB1\*09:08 alleles. Specific PCR fragment of 245 bp in the DRB1\*03:68N allele.

Primer mix 15: Specific PCR fragment of 95 bp in the DRB1\*03:112 allele. Specific PCR fragment of 155 bp in the DRB1\*03:17, 03:37, 03:62 and 03:80 and the DRB1\*11:39, 11:43, 11:50, 11:115, 11:145, 11:151, 11:168, 11:171, 11:174, 13:118, 13:131, 13:135, 13:150, 14:59, 14:86, 14:96 and 14:153-14:154 and in the DRB3\*02:54? alleles.

Primer mix 16: Specific PCR fragment of 50 bp in the DRB1\*03:14-03:15:02 and 03:90 and the DRB1\*04:98:01-04:98:02, 11:105, 14:107 and 14:111 alleles. Specific PCR fragment of 215 bp in the DRB1\*03:132 allele.

Primer mix 17: Specific PCR fragment of 100 bp in the DRB1\*03:93 and the DRB1\*13:125 alleles. Specific PCR fragment of 230 bp in the DRB1\*03:34 allele. Specific PCR fragment of 255 bp in the DRB1\*03:20 and the DRB1\*11:06:01-11:06:03, 11:21, 11:47, 11:57, 11:129, 11:174, 13:58, 13:81, 13:163, 14:29 and 14:99 alleles.

Primer mix 19: Specific PCR fragment of 150 bp in the DRB1\*03:124 allele. Specific PCR fragment of 175 bp in the DRB1\*03:22 allele.

Primer mix 20: Specific PCR fragment of 75 bp in the DRB1\*03:25, 03:31, 03:75 and 03:118 and the DRB1\*11:80, 11:135 and 11:142 alleles. Specific PCR fragment of 120 bp in the DRB1\*03:28 allele. Specific PCR fragment of 245 bp in the DRB1\*03:68N allele.

Primer mix 21: Specific PCR fragment of 70 bp in the DRB1\*03:21, 03:30 and 03:43 and the DRB1\*11:10:01-11:10:02, 11:12:01-11:13:02, 11:114, 11:179, 11:190-11:191, 11:196, 11:202, 12:05, 12:14-12:15, 12:56, 13:57, 13:64, 13:83, 13:107, 13:175, 13:195, 13:204, 14:35, 14:65, 14:72, 14:138 and 14:175 alleles. Specific PCR fragment of 150 bp in the DRB1\*03:42 and 03:87 and the DRB1\*11:30, 13:67 and 13:195 and in the DRB3\*02:32 alleles. Specific PCR fragment of 70 bp and 150 bp in the DRB3\*01:23 allele.

Primer mix 22: Specific PCR fragment of 95 bp in the DRB1\*03:18 allele. Specific PCR fragment of 215 bp in the DRB1\*03:48 allele.

Primer mix 23: Specific PCR fragment of 65 bp in the DRB1\*03:09 and 03:23 alleles. Specific PCR fragment of 165 bp in the DRB1\*03:19 and in the DRB3\*01:31 alleles.

Primer mix 25: Specific PCR fragment of 80 bp in the DRB1\*03:13:01-03:13:02, 03:67N and 03:88 and in the DRB3\*01:01:03 and DRB3\*02:19 alleles. Specific PCR fragment of 180 bp in the DRB1\*03:32 allele. Specific PCR fragment of 230 bp in the DRB1\*03:69 allele.

Primer mix 27: Specific PCR fragment of 115 bp in the DRB1\*03:43 and 03:71:01-03:71:02 and the DRB1\*11:15, 11:76, 11:114, 11:183, 11:196, 13:34, 13:62, 13:64, 13:136, 13:151, 13:174, 13:204, 13:221, 14:41, 14:77 and 14:110 and in the DRB3\*01:14 and 01:25? alleles. Specific PCR fragment of 150 bp in the DRB1\*03:53 and the DRB1\*14:150 alleles. Specific PCR fragment of 175 bp in the DRB1\*03:46 and 03:130 alleles. Specific PCR fragment of 225 bp in the DRB1\*03:11:01, 03:17, 03:24, 03:27, 03:34-03:35 and 03:81 and the DRB1\*11:53 and 11:136 and in the DRB3\*02:54? alleles.

Primer mix 28: Specific PCR fragment of 75 bp in the DRB1\*03:67N allele. Specific PCR fragment of 160 bp in the DRB1\*03:36 allele. Specific PCR fragment of 195 bp in the DRB1\*03:45 allele.

Primer mix 29: Specific PCR fragment of 155 bp in the DRB1\*03:40-03:41:02, 03:52, 03:75, 03:105 and 03:118 and the DRB1\*11:07:01-11:07:02, 11:103:01-11:103:02, 11:105, 11:107, 11:125 and 11:173 alleles. Specific PCR fragment of 180 bp in the DRB1\*03:39 allele. Specific PCR fragment of 215 bp in the DRB1\*03:92 and the DRB1\*14:76 and 14:79 alleles.

Primer mix 31: Specific PCR fragment of 205 bp in the DRB1\*03:45 and 03:92 and the DRB1\*14:76 and 14:79 alleles. Specific PCR fragment of 245 bp in the DRB1\*03:50 allele.

**4**The DRB1\*03 primer set cannot separate the DRB1\*03:77, 03:84, 03:89, 03:95 and the DRB1\*13:144 alleles. These alleles can be distinguished by the DR low resolution kit and/or the DRB1\*13 high resolution kit.

The DRB1\*03 primer set cannot separate the DRB1\*03:97 and the 14:33 alleles. These alleles can be distinguished by the DR low resolution kit and/or the DRB1\*14 high resolution kit.

The DRB1\*03 primer set cannot separate the DRB1\*03:126 and the 14:95 alleles. These alleles can be distinguished by the DR low resolution kit and/or the DRB1\*14 high resolution kit.

**5**The following DRB1\*03 alleles can be distinguished by the different sizes of the specific PCR product:

|  |  |
| --- | --- |
| **Alleles** | **Primer mix** |
| DRB1\*03:18, 03:48 | 22 |
| DRB1\*03:20, 03:93 | 17 |

**6**This lot of the DRB1\*03 kit cannot distinguish the DRB1\*03:55 or DRB1\*03:58 and the DRB1\*03:01:01:01-03:01:25, 03:56, 03:59, 03:61, 03:66, 03:70, 03:73, 03:78, 03:83, 03:86, 03:91, 03:94, 03:98-03:100:02, 03:104, 03:106-03:109, 03:114, 03:116, 03:121, 03:127-03:129, 03:133-03:134 and 03:136 alleles, or the DRB1\*03:08 and DRB1\*03:65 alleles.

The DRB1\*03 subtyping kit cannot distinguish the following silent mutations: the DRB1\*03:01:01:01-03:01:25, the DRB1\*03:02:01-03:02:03, the DRB1\*03:04:01-03:04:02, the DRB1\*03:05:01-03:05:03, the DRB1\*03:13:01-03:13:02, the DRB1\*03:41:01-03:41:02 or the DRB1\*03:71:01-03:71:02 alleles.

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

“w”, may be weakly amplified.

Change in revision R01 compared to R00:

1. The Interpretation Table has been corrected, to include missing amplifications in primer mix 31.

Changes in revision R02 compared to R01:

1. Primer mix 27 does not amplify the DRB1\*03:51, 03:55 and 03:133 alleles. Thus, this lot of the DRB1\*03 kit cannot distinguish the DRB1\*03:55 and the DRB1\*03:01:01:01-03:01:25, 03:56, 03:59, 03:61, 03:66, 03:70, 03:73, 03:78, 03:83, 03:86, 03:91, 03:94, 03:98-03:100:02, 03:104, 03:106-03:109, 03:114, 03:116, 03:121, 03:127-03:129, 03:133-03:134, 03:136 alleles. This has been corrected in the Specificity and Interpretation Tables.

Changes in revision R03 compared to R01:

1. Primer mix 5 does not amplify the DRB1\*03:58 and 03:65 alleles. Thus, this lot of the DRB1\*03 kit cannot distinguish the DRB1\*03:58 and the DRB1\*03:01:01:01-03:01:25, 03:55, 03:56, 03:59, 03:61, 03:66, 03:70, 03:73, 03:78, 03:83, 03:86, 03:91, 03:94, 03:98-03:100:02, 03:104, 03:106-03:109, 03:114, 03:116, 03:121, 03:127-03:129, 03:133-03:134 and 03:136 alleles, or the DRB1\*03:08 and the DRB1\*03:65 alleles. This has been corrected in the Specificity and Interpretation Tables.