Gel Documentation Form and Worksheet

DRB1\*04 (101.114-24/03, -24u/03u) Lot No: 9D2 Expiry Date: 2018-12-01

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

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

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

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

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

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

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

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

|  |
| --- |
| PHOTO DOCUMENT |

**Gel Picture**



‘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 21 and 22 may have tendencies of unspecific amplifications.

Primer mixes 14, 15, 31, 38 and 46 have a tendency to giving rise to primer oligomer formation.

Primer mixes 24 and 32 may give rise to a lower yield of HLA-specific PCR product than the other DRB1\*04 primer mixes.

Primer mix 36 may amplify the DRB1\*11:60, 11:109 and 15:68 alleles.

Primer mix 48 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\*04 alleles in bold lettering are listed as confirmed alleles on the on the IMGT/HLA web page [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla), release 3.23.0, January 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 11: Specific PCR fragment of 175 bp in the DRB1\*04:15, 04:61, 04:138, 04:145, 04:154 and 04:170 and the DRB1\*11:22 alleles. Specific PCR fragment of 220 bp in the DRB1\*04:22 and 04:98:02 alleles.

Primer mix 12: Specific PCR fragment of 180 bp in the DRB1\*04:16 and 04:180 alleles. Specific PCR fragment of 265 bp in the DRB1\*04:23, 04:52, 04:151, 04:203 and 04:207 alleles.

Primer mix 13: Specific PCR fragment of 170 bp in the DRB1\*04:46 allele. Specific PCR fragments of 225 bp in the DRB1\*04:12, 04:18, 04:25, 04:31, 04:54-04:55, 04:58, 04:86, 04:103, 04:126, 04:185 and 04:191 alleles.

Primer mix 15: Specific PCR fragment of 175 bp in the DRB1\*04:123 and the DRB1\*01:10 alleles. Specific PCR fragment of 215 bp in the DRB1\*04:34 allele.

Primer mix 19: Specific PCR fragment of 140 bp in the DRB1\*04:39 allele. Specific PCR fragment of 210 bp in the DRB1\*04:24, 04:69, 04:78, 04:101 and 04:168 and the DRB1\*14:10 and 14:143 alleles.

Primer mix 20: Specific PCR fragment of 100 bp in the DRB1\*04:63, 04:110 and 04:161 alleles. Specific PCR fragment of 175 bp in the DRB1\*04:40 allele. Specific PCR fragment of 255 bp in the DRB1\*04:27 allele.

Primer mix 21: Specific PCR fragment of 155 bp in the DRB1\*04:29 allele. Specific PCR fragment of 210 bp in the DRB1\*04:37, 04:58, 04:73, 04:105:01-04:105:02, 04:122, 04:132 and 04:206 alleles.

Primer mix 23: Specific PCR fragment of 135 bp in the DRB1\*04:33 allele. Specific PCR fragment of 255 bp in the DRB1\*04:201 allele.

Primer mix 25: Specific PCR fragment of 125 bp in the DRB1\*04:26 allele. Specific PCR fragment of 230 bp in the DRB1\*04:45 allele. Specific PCR fragment of 260 bp in the DRB1\*04:64 allele.

Primer mix 27: Specific PCR fragment of 115 bp in the DRB1\*04:10:01-04:12, 04:60, 04:67, 04:91, 04:126, 04:136, 04:147, 04:201, 04:203 and 04:207 and in the DRB1\*03:12, 08:06, 08:10, 08:12, 08:22, 08:54, 08:70, 12:46, 13:04, 13:32, 13:48, 13:58, 13:75, 13:81, 13:89, 13:93-13:94:02, 13:108, 13:164, 13:169, 14:65, 14:78, 14:169-14:170 and 15:12 alleles. Specific PCR fragment of 265 bp in the DRB1\*04:49 allele.

Primer mix 29: Specific PCR fragment of 75 bp in the DRB1\*04:07:01-04:07:05, 04:17:01-04:17:02, 04:20, 04:69, 04:74, 04:92, 04:94:02N, 04:102, 04:129, 04:160, 04:170, 04:172, 04:197 and 04:199 and the DRB1\*01:17, 09:01:02-09:01:05, 09:01:08-09:02:02, 09:04-09:05, 09:07-09:08, 09:10-09:27, 14:07:01-14:07:02, 14:14, 14:36, 14:42, 14:44:01-14:44:03, 14:51, 14:68:01-14:68:02, 14:93, 14:134 and 14:143 and in the DRB5\*01:12 alleles. Specific PCR fragment of 260 bp in the DRB1\*04:64 allele.

Primer mix 31: Specific PCR fragment of 85 bp in the DRB1\*04:14, 04:106 and 04:132 and the DRB1\*01:03, 01:39N, 08:40, 11:11:01, 11:11:03, 11:14:01-11:14:02, 11:20, 11:68, 11:73, 11:93, 11:122, 11:168, 11:184, 13:02:01-13:02:03, 13:02:05-13:02:09,13:02:11-13:03:06, 13:23:01-13:23:02, 13:29, 13:31, 13:33:01-13:34, 13:36-13:39, 13:41, 13:45, 13:63, 13:65-13:67, 13:73-13:74, 13:85, 13:88, 13:90, 13:95-13:97:02, 13:99, 13:101, 13:103-13:104, 13:107, 13:115, 13:120, 13:122-13:124, 13:126, 13:128, 13:135, 13:139, 13:143, 13:145, 13:147, 13:151-13:152, 13:155, 13:159, 13:165, 13:167-13:168, 13:170-13:172, 13:174, 13:179-13:180, 13:182, 13:188, 13:194-13:195, 13:198, 13:202 and 13:207-13:212 alleles. Specific PCR fragment of 260 bp in the DRB1\*04:64 allele.

Primer mix 35: Specific PCR fragment of 210 bp in the DRB1\*04:81N allele. Specific PCR fragment of 245 bp in the DRB1\*04:51 and 04:70 alleles.

Primer mix 36: Specific PCR fragment of 120 bp in the DRB1\*04:96 allele. Specific PCR fragment of 180 bp in the DRB1\*04:71 allele.

Primer mix 38: Specific PCR fragment of 140 bp in the DRB1\*04:89 allele. Specific PCR fragment of 225 bp in the DRB1\*04:73, 04:75, 04:105:01-04:105:02, 04:122 and 04:178N alleles.

Primer mix 39: Specific PCR fragment of 100 bp in the DRB1\*04:83 allele. Specific PCR fragment of 175 bp in the DRB1\*04:15, 04:61-04:62, 04:69, 04:138, 04:145-04:146, 04:154 and 04:170 and the DRB1\*11:22, 14:10, 14:57 and 14:143 alleles.

Primer mix 40: Specific PCR fragment of 145 bp in the DRB1\*04:30 and 04:85 alleles. Specific PCR fragment of 200 bp in the DRB1\*04:38, 04:56:01-04:56:02, 04:88, 04:100, 04:107, 04:125 and 04:205 alleles.

Primer mix 41: Specific PCR fragment of 165 bp in the DRB1\*04:76 and 04:108 alleles. Specific PCR fragment of 205 bp in the DRB1\*04:182 allele.

Primer mix 44: Specific PCR fragment of 165 bp in the DRB1\*04:127 allele. Specific PCR fragment of 240 bp in the DRB1\*04:87, 04:94:01N-04:94:02N, 04:120N, 04:129, 04:157N and 04:186N alleles.

Primer mix 47: Specific PCR fragment of 140 bp in the DRB1\*04:117 allele. Specific PCR fragment of 250 bp in the DRB1\*04:119N and 04:142N alleles.

**4**The following DRB1\*04 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |  |  |
| --- | --- | --- | --- |
| **Alleles** | **Primer mix** | **Alleles** | **Primer mix** |
| DRB1\*04:16, 04:151 | 12 | DRB1\*04:39, 04:101 | 19 |
| DRB1\*04:23, 04:180 | 12 | DRB1\*04:89, 04:178N | 38 |
| DRB1\*04:27, 04:110 | 20 | DRB1\*04:117, 04:119N | 47 |

The DRB1\*04 subtyping kit cannot distinguish the silent mutation in the DRB1\*04:01:01-04:01:16 alleles, the DRB1\*04:02:01-04:02:05 alleles, the DRB1\*04:03:01-04:03:07 and 04:03:09-04:03:11 alleles, the DRB1\*04:04:01-04:04:03 and 04:04:06-04:04:10 alleles, the DRB1\*04:05:01, 04:05:03-04:05:07 and 04:05:09-04:05:11, 04:05:13-04:05:14 and 04:05:16-14:05:17 alleles, the 04:05:02 and 04:05:15 alleles, the DRB1\*04:06:01-04:06:06 alleles, the DRB1\*04:07:01-04:07:05 alleles, the DRB1\*04:08:01-04:08:04 alleles, the DRB1\*04:10:01 and 04:10:03 alleles, the DRB1\*04:11:01-04:11:02 alleles, the DRB1\*04:17:01-04:17:02 alleles, the DRB1\*04:56:01-04:56:02 alleles and the DRB1\*04:105:01-04:105:02 alleles.

5This lot of the DRB1\*04 subtyping kit cannot distinguish the DRB1\*04:04:01-04:04:03, 04:04:06-04:04:10, 04:118, 04:121, 04:149, 04:177 and 04:193 alleles and the DRB1\*04:79 allele.

‘w’, might be weakly amplified.

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

Changes in revision R01 compared to R00:

1. Primer mix 42 does not amplify the DRB1\*04:79 allele. Thus, this lot of the DRB1\*04 subtyping kit cannot distinguish the DRB1\*04:04:01-04:04:03, 04:04:06-04:04:10, 04:118, 04:121, 04:149, 04:177 and 04:193 alleles and the DRB1\*04:79 allele. This has been corrected in the Specificity and Interpretation Tables.