Gel Documentation Form and Worksheet

HLA-B\*44 (101.563-24/03, -24u/03u) Lot No: 8F4 Expiry Date: 2020-05-01

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 mix 36 has a tendency giving rise to primer oligomer formation.

Primer mixes 25, 29, 37, 45, 52, 55 and 57 may have tendencies of unspecific amplifications, most pronounced in primer mix 45.

Primer mix 39 may give rise to a lower yield of HLA-specific PCR product than the other B\*44 primer mixes.

In primer mix 42 the positive control band may be weaker than for other HLA-B\*44 primer mixes.

We assume that the fourth exon nucleotide sequences are conserved within allelic groups. Primer mix 25 amplifies the B\*44:27 and B\*44:31 alleles. The purpose of primer mixes 23 and 25 is to distinguish e.g. the B\*44:02,44:02 and B\*44:02,44:27 genotypes.

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-B\*44 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 3.29.0, August 2017.

**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**The following HLA-B\*44 primer mixes have two or more product sizes:

|  |  |  |  |
| --- | --- | --- | --- |
| Primer Mix | Size of spec. PCR product | Amplified HLA-B\*44 alleles | Other amplified HLA Class I alleles |
| **5** | 75 bp  255 bp | \*44:04, 44:28:01-44:28:02, 44:56N, 44:76  \*44:70 | \*07:20, 07:140, 07:153, 35:45, **C\*05:102, C\*14:61** |
| **6** | 170 bp  195 bp | \*44:39  \*44:05:01-44:05:04, 44:10, 44:15, 44:17-44:18, 44:25, 44:31, 44:43:01-44:43:02, 44:58N, 44:62, 44:70, 44:75, 44:77-44:78, 44:82, 44:107, 44:117, 44:123, 44:134, 44:136, 44:139-44:140, 44:144, 44:158, 44:184, 44:213, 44:226, 44:230, 44:236, 44:250-44:251, 44:256, 44:263-44:264 | \*07:20, 07:140, 07:153, 15:14, 15:91, 15:131, 15:161, 18:56, 35:45, 35:71, 35:169, 35:309, 40:132, 40:178, 45:01:01-45:02, 45:05-45:07, 45:09, 45:11-45:20, 46:17, 49:20, 50:02, 51:23, 51:108, 52:15, 53:22, 57:07, 58:07, 82:01-82:03, **C\*02:82, C\*05:102, C\*06:165, C\*08:93, C\*14:61, C\*15:25** |
| **9** | 120 bp  195 bp | \*44:23N  \*44:10, 44:15, 44:18, 44:140 | \*45:01:01-45:01:02, 45:05-45:07, 45:11-45:20, 49:20, 50:02, 82:01-82:03 |
| **10** | 125 bp  150 bp  175 bp | \*44:24, 44:181  \*44:92  \*44:237N |  |
| **18** | 115 bp  155 bp  260 bp | \*44:31, 44:169, 44:182, 40:132, 40:178  \*44:74  \*44:19N, 44:82, 44:96, 44:153 | \*57:07 |
| **19** | 150 bp  190 bp | \*44:11  \*44:30 |  |
| **21** | 100 bp  180 bp | \*44:32  \*44:16, 44:21, 44:64:01-44:64:02, 44:124, 44:150 | \*27:148  \*07:28, **C\*02:97** |
| **22** | 205 bp  290 bp | \*44:177, 44:228  \*44:26 | \*13:81, 35:183, 51:209  \*35:138, 47:07 |
| **26** | 100 bp  145 bp  225 bp | \*44:69:01-44:69:02  \*44:33, 44:81  \*44:85 | **\***38:51, **A\*24:370N**  \*51:226, **A\*24:137,** **A\*32:96** |
| **27** | 160 bp  255 bp | \*44:02:10, 44:34:01  \*44:15, 44:18, 44:20, 44:47, 44:100, 44:197 | \*15:32:02, 40:01:11, 40:58, 49:03, 52:01:02:01-52:01:02:03, 52:01:04, 52:01:09, 52:02:01, 52:03, 52:06:01-52:06:02, 52:09, 52:10:03, 52:21, 52:31:02  \*45:01:01-45:03, 45:05-45:07, 45:09, 45:11-45:20, 49:20, 50:02, 51:23, 51:108, 52:15 |
| **28** | 130 bp  165 bp | \*44:68  \*44:35 |  |
| **29** | 90 bp  255 bp | \*44:117, 44:251  \*44:15, 44:55, 44:103, 44:188, 44:212 | **\***14:10, 35:59:01, 35:242, 37:19:01, 38:20, 39:42, 39:106, **C\*15:05:08, C\*15:140, C\*17:01:10**  \*13:23, 13:55, 18:09, 27:01, 27:142, 37:10, 40:47, 40:96, 40:157, 40:201, 49:02 |
| **31** | 110 bp  195 bp  230 bp  260 bp | \*44:94  \*44:17, 44:43:01-44:43:02, 44:144, 44:230, 44:256  \*44:98  \*44:99 | **\***15:14, 15:91, 15:131, 15:161, 18:56, 35:45, 35:71, 45:09, 46:17, 53:22, 58:07, **C\*02:82, C\*06:165, C\*14:61**  **A\*02:432, A\*30:29, A\*31:61** |
| **33** | 165 bp  195 bp | \*44:81  \*44:16, 44:37:01-44:37:02, 44:64:01-44:64:02, 44:80, 44:91, 44:132, 44:150, 44:190, 44:268 | \*07:28, 08:87, 15:77, 15:233, 52:26, 57:04:01-57:04:02, **A\*23:31, A\*24:106, C\*01:115, C\*02:97, C\*07:231, C\*14:79, C\*16:10** |
| **35** | 85 bp  185 bp | \*44:63  \*44:04, 44:56N, 44:132, 44:137, 44:163, 44:190, 44:268 | \*08:87, **C\*01:115, C\*07:231, C\*14:79, C\*16:10** |
| **36** | 145 bp  235 bp | \*44:40, 44:44, 44:130, 44:156, 44:210  \*44:142 | \*07:149, 08:02, 08:117, 13:97, 15:115, 27:01, 27:142, 38:02:01-38:02:06, 38:03-38:04, 38:08, 38:15, 38:18, 38:23, 38:29, 38:35, 38:43-38:50, 38:62, 38:64, 56:07, 59:08 |
| **40** | 235 bp  260 bp | \*44:10, 44:15, 44:18, 44:48, 44:256  \*44:31, 44:41:01-44:41:02, 44:54, 44:65, 44:106, 44:135, 44:158, 44:184, 44:213, 44:230, 44:236 | \*35:45, 35:71, 35:169, 35:309, 45:01:01-45:03, 45:05-45:07, 45:09, 45:11-45:20, 49:20, 50:02, 53:22, 58:07, 82:01-82:03, **C\*02:82, C\*05:102, C\*06:165, C\*08:93, C\*14:61, C\*15:25**  **\***07:20, 07:140, 07:153, 15:14, 15:91, 15:131, 15:161, 18:56, 27:47, 27:50:01-27:50:02, 40:132, 40:178, 46:17, 58:07, 82:01-82:03, **C\*02:82, C\*05:102, C\*06:165, C\*08:93, C\*14:61** |
| **42** | 130 bp  165 bp | \*44:90, 44:97  \*44:14, 44:62, 44:166, 44:213 | \*40:166, 45:06, **C\*07:69, C\*08:68**  \*08:01:01:01-08:01:22, 08:01:24-08:05, 08:08N-08:12:03, 08:15-08:19N, 08:21-08:24, 08:26:01-08:27, 08:29-08:36, 08:38-08:39, 08:41-08:48, 08:50-08:54:02, 08:56:01-08:69, 08:71-08:73, 08:75-08:76, 08:78, 08:80-08:86N, 08:88, 08:90-08:106, 08:109-08:110, 08:112-08:142, 08:144-08:155, 08:157-08:163, 08:165-08:170, 08:172-08:184, 15:83, 35:87, 37:12, 38:30, 40:136, 40:231, 40:279, 41:01:01-41:03:02, 41:05-41:09, 41:11-41:17, 41:19-41:20, 41:22-41:48, 41:50, 42:01:01-42:02:01:02, 42:04-42:08, 42:10-42:15, 42:17-42:18, 42:21-42:24, 45:14, 51:08:01-51:08:02, 51:20, 51:36, 51:44N, 51:97, 51:141, 51:153, 52:19, 55:20, 55:56, 56:13, 57:09, 82:01-82:03 |
| **43** | 110 bp  165 bp | \*44:52N, 44:115  \*44:29, 44:89 |  |
| **44** | 85 bp  160 bp | \*44:53:01-44:53:02, 44:182, 44:192:01-44:192:02  \*44:73, 44:76, 44:79:01-44:79:02, 44:146, 44:150 | \*07:28, 08:87, **C\*01:115, C\*02:97, C\*07:231, C\*14:79, C\*16:10**  \*07:28, 15:77, 15:233, 52:26, **C\*01:115, C\*02:97, C\*14:79** |
| **45** | 220 bp  250 bp  285 bp | \*44:36  \*44:54, 44:86, 44:106, 44:135, 44:158, 44:184, 44:213  \*44:173 | \*07:20, 07:140, 07:153, 52:56, 53:27 |
| **46** | 175 bp  450 bp | \*44:66, 44:267N  \*44:56N | \*51:11N  \*40:337N |
| **47** | 65 bp  115 bp  140 bp  195 bp | \*44:93, 44:139  \*44:72, 44:204  \*44:191  \*44:58N |  |
| **48** | 105 bp  140 bp  175 bp  260 bp  595 bp | \*44:61N  \*44:59:01-44:59:02, 44:136, 44:191  \*44:237N  \*44:78, 44:120, 44:195N  \*44:59:01-44:59:02, 44:136 | \*35:223  \*35:204, **C\*03:147** |
| **50** | 95 bp  160 bp  195 bp  285 bp | \*44:111, 44:200  \*44:171N  \*44:137, 44:227  \*44:148 | \*58:76, **C\*03:251, C\*03:314, C\*05:10, C\*05:148, C\*08:44, C\*08:61, C\*08:82, C\*08:126, C\*15:130** |
| **51** | 110 bp  200 bp  240 bp | \*44:115  \*44:217N  \*44:259 |  |
| **52** | 130 bp  285 bp  405 bp | \*44:143, 44:261  \*44:173  \*44:118 | \*39:46 |
| **53** | 210 bp  240 bp | \*44:122  \*44:259 |  |
| **54** | 175 bp  270 bp | \*44:253  \*44:126:01-44:126:02, 44:159, 44:195N | **C\*03:337**  \*15:394, 35:247, 58:45:01-58:45:02, **C\*03:96, C\*15:52** |
| **56** | 110 bp  250 bp  280 bp | \*44:198N  \*44:160Q  \*44:104, 44:148 | **C\*03:265N**  **\***35:333Q, 37:16Q, **C\*15:96Q**  \*58:76, **C\*03:251, C\*03:314, C\*05:10, C\*05:148, C\*08:44, C\*08:61, C\*08:82, C\*08:126, C\*15:130** |
| **58** | 210 bp  245 bp | \*44:87-44:88, 44:223  \*44:271 |  |
| **61** | 210 bp  255 bp | \*44:130, 44:156, 44:221, 44:224, 44:258  \*44:15, 44:55, 44:103, 44:188, 44:212 | \*07:149, 08:02, 08:117, 15:115, 18:09, 37:10, 38:02:01-38:02:06, 38:03-38:04, 38:08, 38:15, 38:18, 38:23, 38:29, 38:35, 38:43-38:45, 38:47-38:50, 38:62, 38:64  \*13:23, 13:55, 18:09, 27:01, 27:142, 37:10, 40:47, 40:96, 40:157, 40:201, 49:02 |
| **62** | 115 bp  175 bp  300 bp | \*44:38  \*44:253  \*44:71 | \*13:80, 35:326  **C\*03:337**  **C\*15:141** |

**4**The following HLA-B\*44 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |  |  |
| --- | --- | --- | --- |
| **Alleles** | **Primer mix** | **Alleles** | **Primer mix** |
| B\*44:58N, 44:139 | 47 | B\*44:78, 44:136 | 48 |
| B\*44:69:01, 44:85 | 26 | B\*44:79:01-44:79:02, 44:192 | 44 |
| B\*44:72, 44:93 | 47 | B\*44:94, 44:98 | 31 |
| B\*44:74, 44:169 | 18 | B\*44:160Q, 44:198N | 56 |

‘w’, might be weakly amplified.

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

Change in revision R01 compared to R00:

1. Primer mix 43 amplifies the B\*44:115 allele. This has been corrected in the Specificity and Interpretation Tables.