Gel Documentation Form and Worksheet

HLA-B\*08 (101.513-24/04 -24u/04u) Lot No: 3E6 Expiry Date: 2019-03-01

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 7 and 44 may have a tendency of giving rise to primer oligomer formation.

Primer mixes 8 and 23 may give rise to a lower yield of HLA-specific PCR product than the other B\*08 primer mixes.

Primer mixes 39 and 43 may have tendencies of unspecific amplifications.

Primer mix 47 may give rise to a lower yield of HLA-specific PCR product than the other HLA-B\*08 primer mixes in the B\*08:03 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**HLA-B\*08 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 3.24.0, April 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 5: Specific PCR fragment of 205 bp in the B\*08:148N allele. Specific PCR fragment of 245 bp in the B\*08:03, 08:23, 08:52, 08:54, 08:78, 08:110 and 08:133 and the B\*15:142, 35:218, 35:256, 40:328, 49:25, 51:68, 51:176, 57:49 and in the C\*06:147 alleles.

Primer mix 7: Specific PCR fragment of 215 bp in the B\*08:05 allele. Specific PCR fragment of 250 bp in the B\*08:03, 08:36 and 08:78 and the B\*49:25, 51:68, 51:176, and 57:49 and in the C\*07:377 alleles.

Primer mix 9: Specific PCR fragment of 115 bp in the B\*08:49 and 08:71 and the B\*13:46, 15:83, 27:47, 27:127, 37:01:01-37:01:11, 37:03N-37:06:02, 37:08, 37:10-37:11, 37:13-37:34, 37:36-37:38, 37:41-37:55, 37:57, 38:30, 41:24, 42:10, 42:17, 44:02:01:01-44:02:40, 44:05:01-44:06, 44:08-44:09, 44:11-44:12, 44:14-44:16, 44:18-44:25, 44:27:01-44:27:02, 44:33-44:34:02, 44:41:01-44:42, 44:44, 44:48-44:49, 44:52N, 44:55, 44:58N-44:59:02, 44:62-44:63, 44:66-44:68, 44:70-44:73, 44:75, 44:78, 44:80, 44:83-44:84:02, 44:86-44:91, 44:93, 44:95, 44:97, 44:99-44:102, 44:104, 44:106, 44:112-44:113, 44:116-44:119, 44:126:01-44:127, 44:131-44:134, 44:136-44:140, 44:142, 44:145, 44:148-44:149N, 44:151-44:152, 44:158, 44:162, 44:168-44:173, 44:176-44:177, 44:179, 44:185, 44:187, 44:190-44:191, 44:195N-44:196, 44:200-44:201, 44:206, 44:208, 44:211-44:214, 44:216-44:221, 44:225-44:226, 44:229, 44:235-44:236, 44:238, 44:240, 45:01:01-45:08, 45:10-45:18, 51:08:01-51:08:02, 51:20, 51:36, 51:44N, 51:97, 51:141, 51:153, 52:19, 55:56, 56:13, 57:09, 82:01-82:03 and 83:01 alleles. Specific PCR fragment of 165 bp in the B\*08:07, 08:37, 08:89 and 08:107 and the B\*07:04, 07:19, 07:25, 07:146, 35:260, 37:09, 41:04, 41:18, 41:21, 42:09 and 42:16 alleles. Specific PCR fragment of 115 bp and 165 bp in the B\*15:51, 15:179:01-15:179:02, 15:199, 15:218Q, 18:56, 18:113, 35:38, 35:115, 35:169, 37:35, 37:56, 44:17, 44:51, 44:123, 44:230, 45:09, 53:22 and 57:24 alleles.

Primer mix 10: Specific PCR fragment of 85 bp in the B\*08:40 and the B\*27:83 and 41:19 alleles. Specific PCR fragment of 170 bp in the B\*08:08N allele.

Primer mix 13: Specific PCR fragment of 85 bp in the B\*08:11 allele. Specific PCR fragment of 290 bp in the B\*08:49, 08:60, 08:76 and 08:129 and the B\*42:13 and 53:15 alleles.

Primer mix 14: Specific PCR fragment of 115 bp in the B\*08:43 allele. Specific PCR fragment of 280 bp in the B\*08:12:01-08:12:03, 08:16, 08:49, 08:89, 08:107 and 08:129, and the B\*41:03:01-41:03:02, 42:13 and 53:15 alleles.

Primer mix 18: Specific PCR fragment of 425 bp in the B\*08:15 and the B\*07:13, 07:15, 07:92, 07:253w, 39:27, 40:73, 67:02 and 73:01-73:02 alleles. Specific PCR fragment of 470 bp in the B\*08:39 and the B\*07:93 and 38:27 alleles.

Primer mix 19: Specific PCR fragment of 245 bp in the B\*08:44 allele. Specific PCR fragment of 415 bp in the B\*08:16, 08:123 and 08:145 and the B\*07:13, 07:15, 07:160, 42:18 and 67:02 alleles.

Primer mix 20: Specific PCR fragment of 140 bp in the B\*08:22 allele. Specific PCR fragment of 205 bp in B\*08:17, 08:38, 08:54 and 08:148N and the B\*15:142, 40:328, 44:49, 44:156 and 49:25 alleles.

Primer mix 22: Specific PCR fragment of 130 bp in the B\*08:19N allele. Specific PCR fragment of 215 bp in the B\*08:109 allele.

Primer mix 23: Specific PCR fragment of 85 bp in the B\*08:21 and 08:25 and the B\*41:17 and 53:15 alleles. Specific PCR fragment of 235 bp in the B\*08:17 and 08:47 and the B\*13:39, 13:67, 15:57, 15:135, 35:27, 35:56, 35:185, 35:203, 44:75, 55:12, 56:41, 78:06 and 78:09 and in the C\*05:30 and C\*06:125 alleles.

Primer mix 24: Specific PCR fragment of 85 bp in the B\*08:101 and in the C\*06:147 alleles. Specific PCR fragment of 210 bp in the B\*08:26:01-08:26:03, 08:50, 08:94 and 08:128 and the B\*07:08, 07:32, 07:38, 07:207, 07:228, 14:42, 15:08:01-15:08:02, 15:29, 15:56, 15:191, 15:236, 35:01:01:01-35:19, 35:21-35:25, 35:27, 35:29:01-35:49, 35:51-35:62, 35:64:01-35:71, 35:74-35:75, 35:77, 35:79-35:81, 35:83, 35:86-35:94, 35:96-35:134N, 35:136-35:161, 35:163-35:185, 35:187-35:216N, 35:219-35:225, 35:227-35:228, 35:230, 35:232-35:254, 35:257-35:279, 35:281-35:289, 35:291-35:293, 35:295, 35:297-35:314, 38:06-38:07, 39:58, 39:83, 44:06, 44:232, 48:06, 48:31, 51:01:01:01-51:06:03, 51:08:01-51:21, 51:23-51:24:05, 51:26-51:38, 51:40-51:46, 51:48-51:67, 51:69-51:102, 51:104-51:115, 51:117-51:127, 51:129-51:144, 51:146-51:150, 51:152-51:166, 51:168-51:175, 51:177-51:188, 51:190-51:202, 52:06:01-52:06:03, 53:01:01-53:08:02, 53:10-53:16, 53:18-53:27, 53:29-53:37, 53:39, 53:41, 59:01:01:01-59:07, 59:09, 78:01:01-78:02:02, 78:04 and 78:07-78:09 alleles. Specific PCR fragment of 85 bp and 210 bp in the B\*51:39 allele.

Primer mix 25: Specific PCR fragment of 180 bp in the B\*08:49 and 08:71 and the B\*27:83, 42:10 and 42:17 alleles. Specific PCR fragment of 225 bp in the B\*08:28, 08:35, 08:37, 08:89 and 08:107 and the B\*41:30 and 42:09 alleles. Specific PCR fragment of 180 bp and 225 bp in the B\*41:24 and 53:15 alleles.

Primer mix 27: Specific PCR fragment of 140 bp in the B\*08:22 allele. Specific PCR fragment of 215 bp in the B\*08:29 and the B\*07:195 and 40:281 alleles.

Primer mix 28: Specific PCR fragment of 85 bp in the B\*08:31 allele. Specific PCR fragment of 210 bp in the B\*08:66 allele.

Primer mix 29: Specific PCR fragment of 150 bp in the B\*08:32 and 08:133 and the B\*35:218, 35:256, 51:68 and 51:176 and in the C\*07:209 alleles. Specific PCR fragment of 190 bp in the 08:137 allele. Specific fragment of 250 bp in the B\*08:36 and in the C\*07:377 alleles.

Primer mix 33: Specific PCR fragment of 220 bp in the B\*08:55 and the B\*38:19, 39:03, 39:14, 39:24:01-39:24:02, 39:29, 39:37 and 39:76 alleles. Specific PCR fragment of 255 bp in the B\*08:135 and the B\*35:89 alleles. Specific PCR fragment of 285 bp in the B\*08:42 allele.

Primer mix 34: Specific PCR fragment of 210 bp in the B\*08:45 allele. Specific PCR fragment of 305 bp in the B\*08:59:01-08:59:02 and 40:228? alleles.

Primer mix 35: Specific PCR fragment of 120 bp in the B\*08:51 allele. Specific PCR fragment of 205 bp in the B\*08:57 allele. Specific PCR fragment of 235 bp in the B\*08:113 allele.

Primer mix 36: Specific PCR fragment of 125 bp in the B\*08:20, 08:53:01-08:53:02 and 08:84 and the B\*42:19 alleles. Specific PCR fragment of 270 bp in the B\*08:33 and 08:100 alleles.

Primer mix 37: Specific PCR fragment of 155 bp in the B\*08:41 and the B\*35:192, 35:240 and 51:141 alleles. Specific PCR fragment of 190 bp in the B\*08:105 allele. Specific fragment of 270 bp in the B\*08:48 allele.

Primer mix 38: Specific PCR fragment of 180 bp in the B\*08:61 and 08:105 alleles. Specific PCR fragment of 265 bp in the B\*08:58 allele.

Primer mix 39: Specific PCR fragment of 190 bp in the B\*08:46 and 08:137 alleles. Specific PCR fragment of 225 bp in the B\*08:132 and the B\*07:29 and 07:186 and in the C\*02:14:01-02:14:02, 02:107, C\*03:39, 03:67, C\*05:43, C\*06:05, 06:44, 06:124, 06:138, C\*07:39-07:40, 07:177, 07:210, 07:238, 07:328, 07:335, C\*08:37, C\*15:23, 15:63, C\*16:21 and 16:80 alleles.

Primer mix 41: Specific PCR fragment of 180 bp in the B\*08:108 allele. Specific PCR fragment of 210 bp in the B\*08:62 and 08:94 and the B\*07:08, 07:32, 07:38, 07:207, 07:228, 18:07:01-18:07:02, 18:29, 18:79, 27:23, 35:50, 35:84, 35:162, 35:197, 35:217, 35:231, 35:267, 35:280, 37:21, 38:06-38:07, 39:58, 39:83, 40:07-40:08, 40:13, 40:68, 40:106, 40:162, 40:220, 40:232, 41:46, 48:06, 48:31, 51:57, 51:103, 51:116, 51:151 and 53:21 alleles.

Primer mix 43: Specific PCR fragment of 185 bp in the B\*08:64 and the B\*07:113 alleles. Specific PCR fragment of 215 bp in the B\*08:70 and the B\*07:55, 07:100, 15:07:01-15:07:03, 15:45, 15:68, 15:126, 15:207, 15:324, 15:331, 46:12 and 48:19 and in the C\*02:60 alleles.

Primer mix 44: Specific PCR fragment of 170 bp in the B\*08:67N allele. Specific PCR fragment of 215 bp in the B\*08:65 and the B\*14:28 and 39:12 alleles.

Primer mix 45: Specific PCR fragment of 120 bp in the B\*08:68 allele. Specific PCR fragment of 225 bp in the B\*08:49 and 08:69 and the B\*42:06 and 42:20 alleles. Specific PCR fragment of 250 bp in the B\*08:150 allele.

Primer mix 46: Specific PCR fragment of 175 bp in the B\*08:140 allele. Specific PCR fragment of 295 bp in the B\*08:104 allele.

**4**The HLA-B\*08 primer set cannot separate the B\*08:156 and the B\*15:180 alleles. These alleles can be distinguished by the HLA-B low resolution kit and/or the HLA-B\*15 high resolution kit.

**5**The following HLA-B\*08 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Alleles** | **Primer mix** | **Alleles** | | **Primer mix** |
| B\*08:11, 08:76 | 13 | B\*08:45, 08:59:01-08:59:02 | | 34 |
| B\*08:19N, 08:109 | 22 | B\*08:51, 08:57 | | 35 |
| B\*08:21, 08:47 | 23 | B\*08:58, 08:61 | | 38 |
| B\*08:33, 08:53:01-08:53:02 | 36 | B\*08:68, 08:69 | | 45 |
| B\*08:41, 08:48 | 37 | B\*08:101, 08:128 | | 24 |
| B\*08:44, 08:145 | 19 | B\*08:104, 08:140 | 46 | |

The HLA-B\*08 subtyping primer set cannot distinguish the silent mutations in the B\*08:01:01-08:01:34 alleles, the B\*08:12:01-08:12:03 alleles, the B\*08:26:01-08:26:03 alleles, the B\*08:53:01-08:53:02 alleles, the B\*08:56:01-08:56:02 or the 08:59:01-08:59:02 alleles.

**6**This lot of the B\*08 kit cannot distinguish the B\*08:30N and the B\*08:01:01-08:01:34, 08:73, 08:75, 08:77, 08:80-08:81, 08:83, 08:88, 08:90-08:93, 08:96-08:99, 08:106, 08:112, 08:114-08:115, 08:118-08:121, 08:124-08:125, 08:127, 08:130, 08:138, 08:141-08:142, 08:144, 08:147, 08:149, 08:152-08:155 and 08:157-08:161 alleles.

‘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 27 does not amplify the B\*08:30N allele. Thus, this lot of the B\*08 kit cannot distinguish the B\*08:30N and the B\*08:01:01-08:01:34, 08:73, 08:75, 08:77, 08:80-08:81, 08:83, 08:88, 08:90-08:93, 08:96-08:99, 08:106, 08:112, 08:114-08:115, 08:118-08:121, 08:124-08:125, 08:127, 08:130, 08:138, 08:141-08:142, 08:144, 08:147, 08:149, 08:152-08:155 and 08:157-08:161 alleles. This has been corrected in the Specificity and Interpretation Tables.

Changes in revision R02 compared to R01:

1. Primer mix 11 does not amplify the B\*41:06 allele. This has been corrected in the Specificity and Interpretation Tables.