Gel Documentation Form and Worksheet

HLA-B\*13 Lot No: 9E7 Expiry Date: 2019-08-01

(101.515-12/12u)

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 2 and 13 may give rise to a weak band of about 500 bp. This band should be disregarded when interpreting HLA-B\*13 SSP subtypings.

Primer mixes 1, 5 and 24 may give rise to a lower yield of HLA-specific PCR product than the other B\*13 primer mixes.

Primer mixes 7, 8, 12 and 16 may have tendencies of unspecific amplifications.

Primer mix 6 has a tendency to giving rise to primer oligomer formation.

Primer mix 31 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\*13 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 3.26.0, 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 5: Specific PCR fragment of 125 bp in the B\*13:22:01-13:22:02, 13:45 and the B\*15:86, 15:224, 35:60, 46:18, 51:157, 54:01:01-54:02, 54:05N, 54:07-54:08N, 54:10, 54:12-54:13, 54:17-54:19, 54:22-54:32, 54:34-54:37, 55:02:01:01-55:02:10, 55:07, 55:10, 55:12, 55:16, 55:18-55:19, 55:22, 55:26, 55:30, 55:34-55:35, 55:37, 55:39, 55:42-55:43, 55:47-55:48, 55:56-55:57, 55:61-55:63, 55:65, 55:67, 55:69-55:72, 55:77, 55:80, 56:10, 56:19N, 56:23, 59:01:01:01-59:01:01:02 and 59:05-59:09 alleles. Specific PCR fragment of 155 bp in the B\*13:16, 13:20, 13:31 and 13:41 alleles. Specific PCR fragment of 125 bp and 155 bp in the B\*07:78, 15:42, 54:20, 55:01:01-55:01:16, 55:03, 55:05, 55:09, 55:11, 55:15, 55:21, 55:24-55:25, 55:29, 55:31, 55:33, 55:36, 55:38, 55:40, 55:44-55:45, 55:52-55:55N, 55:59-55:60 55:64, 55:66, 55:73-55:76 and 55:78 and in the C\*01:90, C\*15:91 and 15:128 alleles. Specific PCR fragments of 125 bp and 180 bp in the B\*13:03 and the B\*40:71, 44:10, 46:11, 54:03, 56:01:01:01-56:01:04, 56:01:06-56:02:01, 56:04, 56:07-56:08, 56:13-56:14, 56:16-56:17, 56:20:01-56:20:02, 56:24, 56:26-56:30, 56:33-56:36, 56:38-56:49, 56:51, 58:73, 59:04 and 82:01-82:03 alleles. Specific PCR fragments of 125 bp, 155 bp and 180 bp in the B\*13:48 and the B\*15:73, 15:303, 51:62, 51:106, 52:25:01-52:25:02, 54:33, 56:25 and 56:37 alleles. All bands may not always be visible.

Primer mix 9: Specific PCR fragment of 130 bp in the B\*13:42 and the B\*51:38 alleles. Specific PCR fragment of 230 bp in the B\*13:08 allele.

Primer mix 10: Specific PCR fragment of 105 bp in the B\*13:01:11, 13:21 and 13:71 and the B\*07:05:01:01-07:05:07, 07:06:01, 07:32, 07:34, 07:40, 07:53, 07:69, 07:78, 07:80, 07:90, 07:97, 07:105, 07:112, 07:123, 07:137-07:138, 07:140, 07:176, 07:182N, 07:201N, 07:206-07:207, 07:209-07:210, 07:213, 07:222, 07:249, 07:258, 07:264, 07:269-07:270, 07:278, 07:283, 08:01:02, 08:01:13, 08:20, 08:53:02, 08:79, 15:150, 15:188, 27:07:01-27:07:05, 27:11, 27:20, 27:24, 27:27, 27:43, 27:125, 27:130, 35:09:02-35:09:03, 35:18, 35:31, 35:88, 35:230, 38:63, 40:01:11, 40:10:01:01-40:10:02, 40:12-40:13, 40:19, 40:21, 40:47, 40:58, 40:76, 40:96, 40:109-40:110, 40:117, 40:137, 40:149, 40:157, 40:188, 40:201, 40:215, 40:248, 40:282, 40:292, 44:31, 48:01:01-48:01:06, 48:03:01-48:07, 48:09-48:11, 48:13, 48:15-48:16, 48:18-48:22, 48:24, 48:28-48:41, 51:01:09, 51:01:21, 51:01:25, 51:10, 51:24:02-51:24:04, 51:81, 52:54, 54:11, 55:04, 55:08, 55:49, 56:20:02, 58:27, 67:03 and 81:01-81:08 and in the C\*01:90, 01:116, C\*02:68, C\*03:317, C\*15:24 and 15:128 alleles.

Specific PCR fragment of 210 bp in the B\*13:09, 13:39 and 13:67 and the B\*07:177, 08:102, 08:122, 14:48, 15:53, 15:183, 15:212, 15:336, 15:345, 18:80, 18:102, 18:107, 27:08, 27:12, 27:18, 27:26, 27:40, 27:42, 27:44, 27:89, 27:153, 35:63, 35:84, 35:90, 35:150:01-35:150:02, 35:184, 35:201, 35:217, 35:317, 40:01:14, 40:01:21, 40:01:26, 40:01:28, 40:01:30, 40:01:32, 40:01:46, 40:02:10, 40:02:13, 40:02:18, 40:02:20, 40:03, 40:06:05, 40:06:10, 40:09, 40:14:01-40:14:02, 40:18, 40:20, 40:24, 40:26, 40:28, 40:31-40:33, 40:35:02, 40:42, 40:44, 40:52, 40:59-40:60, 40:71-40:72:01, 40:77-40:78, 40:87:01, 40:89, 40:95, 40:98, 40:114:02, 40:120-40:121, 40:124:01-40:124:02, 40:131, 40:136, 40:158-40:159, 40:172, 40:180-40:181, 40:184, 40:187, 40:237-40:238, 40:267, 40:280, 40:284, 40:341, 41:01:01-41:04, 41:06-41:07, 41:09-41:11, 41:13-41:21, 41:23-41:25, 41:27-41:35, 41:37-41:45N, 41:47-41:49, 44:09, 44:46, 44:75, 44:90, 44:129, 44:131, 44:254, 45:01:01-45:15, 45:17-45:19, 46:10, 47:02, 47:03w, 50:01:01-50:02, 50:04-50:20, 50:31-50:49, 56:36 and 73:01-73:02 alleles.

Specific PCR fragments of 105 bp and 210 bp in the B\*27:33 and the B\*40:01:01-40:01:10, 40:01:12-40:01:13, 40:01:15-40:01:20, 40:01:22-40:01:25, 40:01:27, 40:01:29, 40:01:31, 40:01:33-40:01:45, 40:01:47-40:02:09, 40:02:11-40:02:12, 40:02:14-40:02:17, 40:02:19, 40:02:19, 40:02:21-40:02:25, 40:04-40:06:04, 40:06:06-40:06:09, 40:06:11-40:08, 40:11:01-40:11:02, 40:14:03-40:16, 40:22N-40:23, 40:25, 40:27:01-40:27:02, 40:29-40:30, 40:34-40:35:01, 40:36-40:40, 40:43, 40:45-40:46, 40:48-40:51, 40:53-40:57, 40:61-40:70:02, 40:72:02-40:75, 40:79-40:86, 40:87:02-40:88, 40:90-40:94, 40:97, 40:99-40:108, 40:111-40:114:01, 40:115-40:116, 40:118N-40:119, 40:122-40:123, 40:125-40:130:02, 40:132-40:135, 40:138-40:148, 40:150-40:156, 40:160:01-40:171, 40:173-40:179, 40:182-40:183, 40:185-40:186:02, 40:189-40:200, 40:202-40:214, 40:216N-40:236, 40:239-40:247, 40:249-40:266, 40:268-40:279, 40:281, 40:283, 40:285-40:291N, 40:293-40:304, 40:306, 40:308-40:311, 40:313-40:324, 40:326-40:329, 40:331-40:339, 40:342-40:344 and 41:08 alleles. Both bands may not always be visible.

Primer mix 13: Specific PCR fragment of 100 bp in the B\*13:19, 13:52 and 13:57 and the B\*27:04:01-27:04:03, 27:06, 27:20, 27:24-27:25, 27:66N, 27:68-27:69, 27:100, 27:103, 27:105 and 49:34 alleles. Specific PCR fragment of 180 bp in the B\*13:12:01-13:12:02 alleles.

Primer mix 15: Specific PCR fragment of 105 bp in the B\*13:22:01 and the B\*07:78, 15:73, 15:303, 40:48, 54:22, 56:01:03 and 56:20:02 and in the C\*15:91 and 15:128 alleles. Specific PCR fragment of 245 bp in the B\*13:14 and 13:83 and the 07:149, 18:09w, 27:01w, 27:142w, 37:10w, 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, 40:96w, 40:157w, 40:201w, 44:103w, 44:130, 44:212w and 44:224 alleles.

Primer mix 16: Specific PCR fragment of 90 bp in the B\*13:17, 13:33 and 13:57 and the B\*15:399, 35:46 and 35:207 alleles. Specific PCR fragment of 205 bp in the B\*13:15 and the B\*14:37, 49:09, 51:62, 51:106, 52:25:01-52:25:02 and 56:34 alleles.

Primer mix 17: Specific PCR fragment of 160 bp in the B\*13:49N allele. Specific PCR fragment of 220 bp in the B\*13:27 and 13:43 alleles.

Primer mix 18: Specific PCR fragment of 155 bp in the B\*13:28 allele. Specific PCR fragment of 205 bp in the B\*13:32 allele. Specific PCR fragment of 275 bp in the B\*13:44 allele.

Primer mix 19: Specific PCR fragment of 195 bp in the B\*13:34 and in the C\*17:08 alleles. Specific PCR fragment of 240 bp in the B\*13:76N allele. Specific PCR fragment of 315 bp in the B\*13:96 and 39:77 alleles. Specific PCR fragment of 340 bp in the B\*13:38 allele.

Primer mix 21: Specific PCR fragment of 120 bp in the B\*13:29 allele. Specific PCR fragment of 180 bp in the B\*13:30 and 13:40 and the B\*44:219 alleles.

Primer mix 22: Specific PCR fragment of 135 bp in the B\*13:25 allele. Specific PCR fragment of 175 bp in the B\*13:40 allele. Specific PCR fragment of 300 bp in the B\*13:86 and the B\*07:225, 35:02:01-35:02:07, 35:02:08w, 35:81, 35:83, 35:95, 35:129N, 35:146, 35:154, 35:157, 35:162, 35:172, 35:182-35:184, 35:211, 35:220, 35:258, 35:270, 35:273, 35:285, 35:309, 35:311, 35:323, 45:12, 50:36, 53:19, 53:36 and 58:65 alleles.

Primer mix 23: Specific PCR fragment of 130 bp in the B\*13:42 and the B\*51:38 alleles. Specific PCR fragment of 200 bp in the B\*13:37 allele. Specific PCR fragment of 275 bp in the B\*13:44 allele.

Primer mix 25: Specific PCR fragment of 160 bp in the B\*13:70 allele. Specific PCR fragment of 430 bp in the B\*13:64 allele.

Primer mix 26: Specific PCR fragment of 120 bp in the B\*13:50 and the B\*27:82 allele. Specific PCR fragment of 175 bp in the B\*13:47 allele.

Primer mix 27: Specific PCR fragment of 80 bp in the B\*13:96 and the B\*39:77 alleles. Specific PCR fragment of 165 bp in the B\*13:61 allele. Specific PCR fragment of 185 bp in the B\*13:69 allele.

**4**The following HLA-B\*13 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |
| --- | --- |
| **Alleles** | **Primer mix** |
| B\*13:12:01, 13:52 | 13 |
| B\*13:15, 13:33 | 16 |
| B\*13:27, 13:49N | 17 |
| B\*13:34, 13:38 | 19 |

The HLA-B\*13 kit cannot distinguish the silent mutations in the B\*13:01:01-13:01:10 and 13:01:12 alleles, the 13:02:01-13:02:18 alleles, the B\*13:13:01-13:13:02 alleles, the B\*13:26:01-13:26:02 alleles or the B\*13:56:01N-13:56:02N alleles.

**5**This lot of the B\*13 kit cannot distinguish the B\*13:56:01N and the B\*13:02:01-13:02:18, 13:54, 13:65-13:66, 13:68, 13:74-13:75, 13:81-13:82, 13:84-13:85, 13:88-13:89, 13:91 and 13:93-13:94 alleles.

‘w’, may be weakly amplified.

Changes in revision R01 compared to R00:

1. Primer mix 6 does not amplify the B\*13:56:01N-13.56:02N alleles. Thus, this lot of the B\*13 kit cannot distinguish the B\*13:56:01N-13.56:02N and the B\*13:02:01-13:02:18, 13:54, 13:65-13:66, 13:68, 13:74-13:75, 13:81-13:82, 13:84-13:85, 13:88-13:89, 13:91 and 13:93-13:94 alleles. This has been corrected in the Specificity and Interpretation Tables.

Changes in revision R02 compared to R01:

1. Primer mix 6 does amplify the B\*13.56:02N allele. This has been corrected in the Specificity and Interpretation Tables.