Gel Documentation Form and Worksheet

HLA-B\*51 (101.561-24/03, -24u/03u) Lot No: 7E5 Expiry Date: 2019-08-01

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 and 30 may give rise to a lower yield of HLA-specific PCR product than the other HLA-B\*51 primer mixes.

Primer mixes 25, 30, 39, 41, 65, 75 and 81 may have tendencies of unspecific amplifications.

Primer mixes 27, 39, 58, 60 and 77 have a tendency to giving rise to primer oligomer formation.

Primer mix 83 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\*51 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 3.25.0, July 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 7: Specific PCR fragment of 240 bp in the B\*51:156 allele. Specific PCR fragment of 270 bp in the B\*51:06:01-51:06:03, 51:45, 51:62, 51:64, 51:135 and 51:194 and the B\*07:218, 07:220, 08:118, 14:01:01-14:09, 14:11-14:19, 14:21-14:36, 14:38-14:45, 14:47-14:54, 15:37-15:38:02, 15:185, 15:323, 15:335, 15:364, 15:368, 18:01:01:01-18:09, 18:12:01-18:15, 18:17N-18:20, 18:23N-18:34, 18:36-18:38, 18:40-18:55, 18:57, 18:59-18:68, 18:70-18:88, 18:90-18:100, 18:102-18:104, 18:106-18:109, 18:111-18:123, 18:125-18:127, 27:28, 27:62, 39:32, 39:43, 39:48, 40:51, 40:113, 40:163, 40:174, 56:31 and 67:06 and in the C\*01:30, C\*07:516, 07:521, C\*08:51, 08:114 and C\*12:87 alleles.

Primer mix 9: Specific PCR fragment of 85 bp in the B\*51:08:01-51:08:02, 51:20, 51:44N, 51:97, 51:141 and 51:153 and the B\*08:118, 18:113, 37:04:01-37:04:02, 44:190 and 52:19 alleles. Specific PCR fragment of 205 bp in the B\*51:17 and 51:35 alleles.

Primer mix 10: Specific PCR fragment of 160 bp in the B\*51:01:09, 51:01:21, 51:01:25, 51:10, 51:24:02-51:24:04 and 51:197 and the B\*07:78, 07:84, 27:14, 27:81, 27:130, 40:06:01:01-40:06:04, 40:06:06-40:06:15, 40:44, 40:53, 40:70:01-40:70:02, 40:75, 40:83, 40:86, 40:93, 40:96, 40:103, 40:109-40:110, 40:127, 40:131, 40:148, 40:159, 40:161-40:162, 40:165, 40:167, 40:177, 40:190, 40:230, 40:244, 40:256N, 40:268-40:270, 40:275, 40:294-40:295, 40:298, 40:306-40:307, 40:311, 40:313, 40:317-40:318, 40:330, 52:54, 56:20:02 and 73:01-73:02 alleles. Specific PCR fragment of 245 bp in the B\*51:21, 51:36, 51:101, 51:143, 51:153, 51:157 and 51:197 and the B\*08:09, 08:84, 13:46, 13:53, 14:37, 15:42, 15:83, 35:60, 35:276, 39:06:01-39:06:05, 39:34, 39:57, 39:62, 39:64, 39:83, 39:90, 39:109, 41:01:01-41:01:04, 41:05-41:07, 41:09, 41:12, 41:14, 41:16, 41:20-41:22, 41:25-41:26, 41:28-41:30, 41:32-41:35, 41:37, 41:44, 42:04, 45:08, 46:18, 50:45, 52:37, 54:01:01-54:02, 54:04-54:05N, 54:07-54:08N, 54:10, 54:12-54:25, 54:27-54:32, 54:34-54:35, 55:01:01-55:01:06, 55:01:08-55:03, 55:05, 55:07, 55:10-55:13, 55:15-55:21, 55:23, 55:25-55:48, 55:50, 55:52-55:78, 56:23, 59:01:01:01-59:03, 59:05-59:08 and 78:07 and in the C\*12:183 alleles.

Specific PCR fragment of 160 bp and 245 bp in the B\*54:11 and in the C\*07:463 alleles.

Primer mix 15: Specific PCR fragment of 100 bp in the B\*51:14 allele. Specific PCR fragment of 230 bp in the B\*51:19 and 51:101 and the B\*35:276, 39:06:01-39:06:05, 39:57, 39:62, 39:64, 39:83, 39:90 and 39:109 alleles.

Primer mix 16: Specific PCR fragment of 170 bp in the B\*51:15, 51:35, 51:62 and 51:106 and the 13:03, 13:48, 15:73, 15:303, 35:291, 40:71, 44:10, 44:15, 44:18, 44:140, 45:01:01-45:01:02, 45:04-45:07, 45:11-45:19, 46:11, 49:01:01-49:01:07, 49:02-49:03, 49:06, 49:08-49:14, 49:16-49:17, 49:19N-49:43, 50:01:01-50:02, 50:04-50:08, 50:10-50:13, 50:15-50:19, 50:32, 50:34-50:38, 50:40-50:44, 50:46-50:47, 52:25:01-52:25:02, 54:03, 54:33, 56:01:01:01-56:01:04, 56:01:06-56:02:02, 56:04, 56:07-56:08, 56:13-56:14, 56:16-56:17, 56:20:01-56:20:02, 56:24-56:30, 56:33-56:49, 56:51, 58:73, 59:04 and 82:01-82:03 and in the C\*03:19, 03:102 and C\*15:15 alleles. Specific PCR fragment of 195 bp in the B\*51:18 allele.

Primer mix 18: Specific PCR fragment of 140 bp in the B\*51:28 allele. Specific PCR fragment of 220 bp in the B\*51:08:01-51:08:02, 51:20, 51:36, 51:44N, 51:97, 51:141 and 51:153 and the B\*08:09, 08:84, 13:46, 15:83, 41:01:01-41:01:04, 41:05-41:07, 41:09, 41:12, 41:14, 41:16-41:17, 41:20-41:22, 41:25-41:26, 41:28-41:30, 41:32-41:35, 41:37, 41:44, 42:04, 44:15, 44:18, 44:20, 44:100, 45:01:01-45:19, 52:19, 55:20, 55:56 and 56:13 alleles.

Primer mix 20: Specific PCR fragment of 110 bp in the B\*51:26 allele. Specific PCR fragment of 200 bp in the B\*51:185 and the 52:09 alleles.

Primer mix 21: Specific PCR fragment of 130 bp in the B\*51:38 and the B\*13:42 alleles. Specific PCR fragment of 185 bp in the B\*51:27N allele.

Primer mix 25: Specific PCR fragment of 120 bp in the B\*51:38 and 51:142 and the B\*13:42 alleles. Specific PCR fragment of 175 bp in the B\*51:30 allele. Specific PCR fragment of 205 bp in the B\*51:53 and the B\*52:29 and 55:54 alleles.

Primer mix 26: Specific PCR fragment of 105 bp in the B\*51:13:01-51:13:02, 51:37, 51:63:01, 51:92:01-51:92:02, 51:97 and 51:172 and the B\*13:04, 13:46, 13:72, 15:04:01:01-15:04:04, 15:16:01:01-15:16:03, 15:67, 15:95, 15:137, 15:155, 15:222, 15:254, 15:293, 15:310, 15:362, 35:37, 35:235, 35:265, 35:276, 39:06:01-39:06:05, 39:33-39:34, 39:50, 39:57, 39:62, 39:64, 39:83, 39:90, 39:102, 39:109, 40:86, 40:159, 40:330, 41:30, 45:02, 45:09, 46:32, 49:04:01-49:05, 50:09, 50:33, 52:14, 52:39, 54:14, 55:13, 55:23, 55:32, 55:58, 55:68, 56:22, 56:50, 59:03 and 73:01-73:02 and in the C\*07:463 and C\*12:183 alleles. Specific PCR fragment of 155 bp in the B\*51:32 and the B\*27:69, 40:213 and 58:52 alleles.

Primer mix 27: Specific PCR fragment of 140 bp in the B\*51:126 and in the C\*08:114 alleles. Specific PCR fragment of 205 bp in the B\*51:33 and the B\*44:165 and 58:53 alleles.

Specific PCR fragment of 270 bp in the B\*51:04, 51:46, 51:56:01-51:56:03 and 51:139 and the B\*14:10, 18:22, 18:69w, 18:105, 35:21, 35:24:01-35:24:02, 35:81, 35:96, 35:109, 35:157, 35:188, 35:190, 35:233, 35:287, 37:04:01w-37:04:02w, 40:28, 44:190, 44:203, 53:02, 53:06, 53:28, 57:14:01-57:14:02, 58:09 and 58:76 and in the C\*15:39 alleles. Specific PCR fragment of 345 bp in the B\*51:39 and the B\*37:52, 44:24, 44:181 and 81:06? alleles.

Primer mix 28: Specific PCR fragment of 205 bp in the B\*51:33 and the B\*44:165 and 58:53 alleles. Specific PCR fragment of 255 bp in the B\*51:23 and 51:108 and the B\*44:15, 44:18, 44:20, 44:47, 44:100, 44:197, 45:01:01-45:03, 45:05-45:07, 45:09, 45:11-45:19, 49:20, 50:02 and 52:15 alleles.

Primer mix 31: Specific PCR fragment of 105 bp in the B\*51:13:01-51:13:02, 51:37, 51:63:01, 51:92:01-51:92:02, 51:97 and 51:172 and the B\*13:04, 13:46, 13:72, 15:04:01:01-15:04:04, 15:16:01:01-15:16:03, 15:67, 15:95, 15:137, 15:155, 15:222, 15:254, 15:293, 15:310, 15:362, 35:37, 35:235, 35:265, 35:276, 39:06:01-39:06:05, 39:33-39:34, 39:50, 39:57, 39:62, 39:64, 39:83, 39:90, 39:102, 39:109, 40:86, 40:159, 40:330, 41:30, 45:02, 45:09, 46:32, 49:04:01-49:05, 50:09, 50:33, 52:14, 52:39, 54:14, 55:13, 55:23, 55:32, 55:58, 55:68, 56:22, 56:50, 59:03 and 73:01-73:02 and in the C\*07:463 alleles. Specific PCR fragment of 225 bp in the B\*51:05, 51:29, 51:54, 51:61:01-51:61:02, 51:82 and 51:197 and the B\*07:78, 07:84, 13:18, 13:31, 13:41, 15:04:01:01-15:04:04, 15:137, 15:303, 35:265, 39:33, 40:161, 46:32, 49:41, 50:47, 52:21, 52:24, 54:10, 54:20, 54:33, 55:09, 55:21, 55:37, 55:52 and 56:43 alleles. Specific PCR fragment of 105 bp and 225 bp in the C\*12:183 allele.

Primer mix 36: Specific PCR fragment of 110 bp in the B\*51:165 and the B\*15:214, 18:81, 35:250 and 57:29 alleles. Specific PCR fragment of 215 bp in the B\*51:43 allele.

Primer mix 37: Specific PCR fragment of 165 bp in the B\*51:44N allele. Specific PCR fragment of 225 bp in the B\*51:55 allele.

Primer mix 38: Specific PCR fragment of 205 bp in the B\*51:37, 51:45, 51:63:01 and 51:97 and the B\*15:38:01-15:38:02, 15:185, 15:335, 15:364, 15:368, 18:01:01:01-18:09, 18:12:01-18:15, 18:17N-18:20, 18:22-18:25, 18:27-18:34, 18:36-18:40, 18:42-18:55, 18:57, 18:59-18:60, 18:62-18:100, 18:102-18:109, 18:111-18:121, 18:123, 18:125-18:127, 35:21, 35:24:01-35:24:02, 35:188, 35:190, 35:287, 39:43, 52:39, 53:02, 53:06, 53:28, 56:31, 57:14:01-57:14:02, 58:09 and 58:76 and in the C\*07:516, 07:521 and C\*12:87 alleles. Specific PCR fragment of 250 bp in the B\*51:173Q allele.

Primer mix 41: Specific PCR fragment of 225 bp in the B\*51:05, 51:29, 51:54, 51:61:01-51:61:02, 51:82 and 51:197 and the B\*07:78, 07:84, 13:18, 13:31, 13:41, 15:04:01:01-15:04:04, 15:137, 15:303, 35:265, 39:33, 40:161, 46:32, 49:41, 50:47, 52:21, 52:24, 54:10, 54:20, 54:33, 55:09, 55:21, 55:37, 55:52 and 56:43 and in the C\*12:183 alleles. Specific PCR fragment of 305 bp in the B\*51:48 and 51:166 and the B\*15:140 and 15:367 and in the C\*02:83, C\*08:24 and C\*16:90 alleles. Specific PCR fragment of 380 bp in the B\*51:169 allele.

Primer mix 42: Specific PCR fragment of 100 bp in the B\*51:60 allele. Specific PCR fragment of 135 bp in the B\*51:49 allele.

Primer mix 43: Specific PCR fragment of 115 bp in the B\*51:58 allele. Specific PCR fragment of 215 bp in the B\*51:50 allele.

Primer mix 44: Specific PCR fragment of 285 bp in the B\*51:76 and 51:166 and the B\*08:39, 15:140, 35:101:02, 35:195, 35:211, 44:185 and 54:34 alleles. Specific PCR fragment of 385 bp in the B\*51:51 and in the C\*01:83w alleles.

Primer mix 45: Specific PCR fragment of 145 bp in the B\*51:178N allele. Specific PCR fragment of 190 bp in the B\*51:52 allele.

Primer mix 46: Specific PCR fragment of 80 bp in the B\*51:57 and the B\*07:206 alleles. Specific PCR fragment of 170 bp in the B\*51:123 and the B\*55:59 alleles.

Primer mix 48: Specific PCR fragment of 95 bp in the B\*51:37, 51:90, 51:92:01-51:92:02 and 51:197 and the B\*07:84, 13:04, 13:35, 13:72, 15:16:01:01-15:16:03, 15:67, 15:95, 15:155, 15:222, 15:254, 15:293, 15:310, 15:362, 35:37, 35:235, 40:44, 40:159, 41:21, 44:20, 44:47, 44:100, 44:197, 45:09, 49:04:01-49:05, 50:33, 50:39, 52:12, 52:26, 54:14-54:16, 55:23, 55:27, 55:46, 55:68 and 56:50 and in the C\*07:463 alleles. Specific PCR fragment of 220 bp in the B\*51:61:01-51:61:02 and the B\*13:31, 13:41, 15:137, 15:303, 50:47, 52:21, 54:33, 55:21 and 56:43 alleles. Specific PCR fragment of 95 bp and 220 bp in the B\*15:04:01:01-15:04:04, 35:265 and B\*46:32 and in the C\*12:183 alleles.

Primer mix 51: Specific PCR fragment of 85 bp in the B\*51:68 allele. Specific PCR of 135 bp in the B\*51:136 allele. Specific PCR fragment of 215 bp in the B\*51:72 allele.

Primer mix 53: Specific PCR of 135 bp in the B\*51:136 allele. Specific PCR fragment of 165 bp in the B\*51:71 and 51:174:01 alleles. Specific PCR fragment of 260 bp in the B\*51:70 and the B\*52:55 alleles.

Primer mix 54: Specific PCR fragment of 120 bp in the B\*51:74 and the B\*08:155, 35:46-35:47, 35:154, 35:207, 35:217, 35:257, 35:278, 40:07-40:08, 40:13, 40:68, 40:106, 40:162, 40:220, 40:232, 41:46, 44:232 and 53:30 alleles. Specific PCR fragment of 250 bp in the B\*51:124 and 51:156 alleles.

Primer mix 55: Specific PCR fragment of 75 bp in the B\*51:77 allele. Specific PCR fragment of 200 bp in the B\*51:75 allele.

Primer mix 57: Specific PCR fragment of 145 bp in the B\*51:79 and 51:178N alleles. Specific PCR fragment of 210 bp in the B\*51:54 and 51:78:01-51:78:02 and the B\*13:17, 13:33, 15:36, 15:89, 15:256, 15:339, 44:02:10, 44:34:01-44:34:02, 44:83, 44:225, 44:245, 52:20, 53:09, 53:11-53:13, 53:31, 53:36, 53:38, 56:07 and 59:08 alleles.

Primer mix 59: Specific PCR fragment of 120 bp in the B\*51:83 allele. Specific PCR fragment of 240 bp in the B\*51:88 allele.

Primer mix 60: Specific PCR fragment of 85 bp in the B\*51:94 allele. Specific PCR fragment of 190 bp in the B\*51:84 and 51:130 and the 49:12 alleles.

Primer mix 61: Specific PCR fragment of 100 bp in the B\*51:85 and 51:192 and the B\*27:14, 27:81, 44:20, 44:47, 44:100, 44:197, 50:20 and 52:26 alleles. Specific PCR fragment of 210 bp in the B\*51:87, 51:115 and 51:181 and in the C\*07:463 alleles.

Primer mix 62: Specific PCR fragment of 180 bp in the B\*51:86 and 51:132 alleles. Specific PCR fragment of 250 bp in the B\*51:95 and the B\*39:57 alleles.

Primer mix 63: Specific PCR fragment of 70 bp in the B\*51:91 allele. Specific PCR fragment of 200 bp in the B\*51:146 and the B\*13:86, 35:02:01-35:02:07, 35:02:08w, 35:81, 35:83, 35:95, 35:129N, 35:146, 35:154, 35:162, 35:172, 35:182-35:184, 35:211, 35:220, 35:258, 35:270, 35:273, 35:285, 35:309, 35:311, 45:12, 50:36, 53:19, 53:36 and 58:65 alleles.

Primer mix 64: Specific PCR fragment of 115 bp in the B\*51:67 and the B\*35:314 alleles. Specific PCR fragment of 170 bp in the B\*51:66 and 51:89 alleles.

Primer mix 65: Specific PCR fragment of 185 bp in the B\*51:98N allele. Specific PCR fragment of 215 bp in the B\*51:103, 51:116 and 51:151 and the B\*08:62, 18:07:01-18:07:02, 18:29, 18:79, 27:23, 35:50, 35:84, 35:162, 35:217, 35:231, 35:280, 37:21, 40:07-40:08, 40:13, 40:68, 40:106, 40:162, 40:220, 40:232 and 41:46 alleles.

Primer mix 66: Specific PCR fragment of 175 bp in the B\*51:109 and 51:132 alleles. Specific PCR fragment of 210 bp in the B\*51:120 allele.

Primer mix 67: Specific PCR fragment of 110 bp in the B\*51:93 and 51:122 alleles. Specific PCR fragment of 230 bp in the B\*51:100 allele.

Primer mix 68: Specific PCR fragment of 160 bp in the B\*51:134 allele. Specific PCR fragment of 190 bp in the B\*51:105 allele.

Primer mix 69: Specific PCR fragment of 105 bp in the B\*51:147 and in the A\*02:01:33w and A\*23:01:14w alleles. Specific PCR fragment of 225 bp in the B\*51:110N allele.

Primer mix 72: Specific PCR fragment of 90 bp in the B\*51:118N allele. Specific PCR fragment of 145 bp in the B\*51:169 allele. Specific PCR fragment of 170 bp in the B\*51:164 and the B\*39:46w and 44:118w alleles.

Primer mix 73: Specific PCR fragment of 170 bp in the B\*51:127 and 51:174:01 and the B\*55:43 alleles. Specific PCR fragment of 295 bp in the B\*51:149N allele.

Primer mix 76: Specific PCR fragment of 175 bp in the B\*51:168 allele. Specific PCR fragment of 255 bp in the B\*51:152 and 51:173Q alleles.

Primer mix 81: Specific PCR fragment of 185 bp in the B\*51:98N allele. Specific PCR fragment of 215 bp in the B\*51:167 allele.

**4**The following HLA-B\*51 alleles can be distinguished by the different sizes of the specific PCR product:

|  |  |  |  |
| --- | --- | --- | --- |
| **Alleles** | **Primer mix** | **Alleles** | **Primer mix** |
| B\*51:26, 51:142 | 20 | B\*51:70, 51:71 | 53 |
| B\*51:30, 51:142 | 25 | B\*51:74, 51:124 | 54 |
| B\*51:39, 51:126 | 27 | B\*51:75, 51:77 | 55 |
| B\*51:51, 51:76 | 44 | B\*51:83, 51:88 | 59 |
| B\*51:57, 51:123 | 46 | B\*51:84, 51:94 | 60 |
| B\*51:66, 51:67 | 64 | B\*51:86, 51:95 | 62 |
| B\*51:68, 51:72 | 51 | B\*51:127, 51:149N | 73 |

**5**The B\*51:119, 51:176 and 51:179 and the B\*52:06:03 alleles will give rise to identical amplification patterns. These alleles can e.g. be distinguished by the HLA-B low resolution kit and/or the HLA-B\*52 high resolution kit.

The HLA-B\*51 subtyping kit cannot distinguish following silent mutations: the B\*51:01:01:01-51:01:01:02, 51:01:03-51:01:07, 51:01:10-51:01:14, 51:01:16-51:01:20, 51:01:22-51:01:24, 51:01:26-51:01:38, 51:01:41 and 51:01:44-51:01:52 alleles, the B\*51:01:08, 51:01:15 and 51:01:43 alleles, the B\*51:01:21 and 51:01:25 alleles, the B\*51:01:42 and 51:01:53 alleles, the B\*51:02:01 and 51:02:03-51:02:05 alleles, the B\*51:08:01-51:08:02 alleles, the B\*51:09:01 and 51:09:03 alleles, the B\*51:13:01-51:13:02 alleles, the B\*51:24:01 and 51:24:05 alleles, the B\*51:24:02-51:24:04 alleles, the B\*51:56:02-51:56:03 alleles, the B\*51:61:01-51:61:02 alleles, the B\*51:92:01-51:92:02 or the B\*51:158:01-51:158:02 alleles.

‘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 36 does not amplify the B\*07:120 and the B\*40:150 alleles. This has been corrected in the Specificity and Interpretation Tables.