Gel Documentation Form and Worksheet

HLA-B\*15 (101.516-24/04, -24u/04u) Lot No: 0D5 Expiry Date: 2018-May-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 40, 59, 80, 82, 84 and 85 have a tendency to giving rise to primer oligomer formation.

Primer mixes 5, 15, 30, 36, 68, 81, 83 and 95 may have tendencies of unspecific amplifications.

Primer mix 91 may give rise to a long unspecific amplification product of approximately 800 bp. This should be disregarded when interpreting the B\*15 typings.

Primer mixes 66 and 77 may give rise to a lower yield of HLA-specific PCR product than the other HLA-B\*15 resolution primer mixes.

Primer mix 96 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\*15 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 3.21.1, August 2015.

**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 4: Specific PCR fragment of 180 bp in the B\*15:01:01:02N allele. Specific PCR fragment of 390 bp in the B\*15:285 allele.

Primer mix 10: Specific PCR fragment of 300 bp in the B\*15:37-15:38:02, 15:185, 15:323 and 15:335 alleles. Specific PCR fragment of 515 bp in the B\*15:06, 15:27:01-15:27:03, 15:84, 15:109, 15:112, 15:195, 15:327 and 15:344 alleles.

Primer mix 13: Specific PCR fragment of 395 bp in the B\*15:09-15:10:04, 15:18:01-15:18:06, 15:21, 15:23, 15:37, 15:44, 15:51-15:52, 15:66, 15:72, 15:80, 15:90, 15:93, 15:99, 15:108, 15:114-15:115, 15:119, 15:124, 15:133-15:134, 15:153, 15:161, 15:176, 15:186, 15:189, 15:197-15:198, 15:200, 15:221, 15:226N, 15:229, 15:238, 15:243, 15:252, 15:263, 15:275, 15:290, 15:292-15:294N, 15:306-15:307, 15:311-15:314, 15:323, 15:329, 15:335, 15:337-15:338 and 15:354 allele. Specific PCR fragment of 435 bp in the B\*15:79N allele.

Primer mix 14: Specific PCR fragment of 465 bp in the B\*15:09-15:10:04, 15:30, 15:37, 15:45, 15:48, 15:63, 15:83, 15:90, 15:99, 15:150, 15:188, 15:243, 15:248, 15:252, 15:287, 15:312-15:313, 15:323-15:324, 15:329, 15:338 and 15:361 alleles. Specific PCR fragment of 515 bp in the B\*15:110 allele.

Primer mix 16: Specific PCR fragment of 120 bp in the B\*15:116 and 15:124 and the B\*40:52, 40:59, 46:06, 46:43, 48:23 and 57:59 alleles. Specific PCR fragment of 190 bp in the B\*15:12, 15:19, 15:270, 15:298 and 15:304N alleles.

Primer mix 20: Specific PCR fragment of 360 bp in B\*15:105 allele. Specific PCR fragment of 505 bp in the B\*15:16:01-15:16:03, 15:34, 15:62, 15:67, 15:85-15:86, 15:95, 15:137, 15:194, 15:222, 15:254, 15:303 and 15:362 and the B\*46:11, 46:18, 46:33 and 46:53 alleles.

Primer mix 21: Specific PCR fragment of 205 bp in the B\*15:16:01-15:16:03, 15:67, 15:95, 15:222, 15:254 and 15:362 and in the C\*03:158 alleles. Specific PCR fragment of 425 bp in the B\*15:43 allele.

Primer mix 24: Specific PCR fragment of 205 bp in the B\*15:26N allele. Specific PCR fragment of 305 bp in the B\*15:33, 15:72, 15:78:01-15:78:03, 15:107, 15:116, 15:141, 15:150, 15:188, 15:209N, 15:248 and 15:287 and the B\*46:06 and 46:45 alleles.

Primer mix 25: Specific PCR fragment of 315 bp in the B\*15:46, 15:53, 15:106 and 15:212 and in the C\*16:85 alleles. Specific PCR fragment of 385 bp in the B\*15:28 allele.

Primer mix 30: Specific PCR fragment of 320 bp in the B\*15:40, 15:47:01-15:47:02, 15:49, 15:52, 15:114, 15:117, 15:124, 15:138, 15:238 and 15:241 and the B\*46:06, 46:25, 46:30 alleles. Specific PCR fragment of 355 bp in the B\*15:75 and 15:312 alleles.

Primer mix 31: Specific PCR fragment of 320 bp in the B\*15:44, 15:50, 15:69, 15:83, 15:86, 15:93, 15:121, 15:186, 15:188, 15:199, 15:218Q, 15:224, 15:252, 15:265 and 15:323 and the B\*46:03, 46:18, 46:21:01-46:21:02 and 46:29 alleles. Specific PCR fragment of 395 bp in the B\*15:114 and 15:153 alleles.

Primer mix 32: Specific PCR fragment of 275 bp in the B\*15:196 allele. Specific PCR fragment of 350 bp in the B\*15:190N allele. Specific PCR fragment of 380 bp in the B\*15:48, 15:108, 15:136, 15:235, 15:249 and the B\*46:19 alleles.

Primer mix 33: Specific PCR fragment of 200 bp in the B\*15:118 allele. Specific PCR fragment of 255 bp in the B\*15:18:03, 15:42, 15:73, 15:86, 15:224 and 15:303 and the B\*46:11 and 46:18 alleles. Specific PCR fragment of 340 bp in the B\*15:192 and 15:281 alleles.

Primer mix 36: Specific PCR fragment of 360 bp in the B\*15:82 and 15:260 and in the C\*03:186:01 alleles. Specific PCR fragment of 435 bp in the B\*15:36, 15:89, 15:115, 15:256 and 15:339 and in the C\*16:85 alleles.

Primer mix 39: Specific PCR fragment of 315 bp in the B\*15:60 and 15:119 and in the C\*07:213 alleles. Specific PCR fragment of 385 bp in the B\*15:95 and 15:285 alleles.

Primer mix 40: Specific PCR fragment of 220 bp in the B\*15:71 and B\*15:175 and in the C\*06:77 alleles. Specific PCR fragment of 425 bp in the B\*15:13:01-15:13:02, 15:16:01-15:17:03, 15:23-15:24:02, 15:67, 15:95, 15:157, 15:162, 15:168, 15:177, 15:196, 15:208, 15:216, 15:222, 15:230, 15:254, 15:268, 15:273, 15:356 and 15:361-15:362 alleles.

Primer mix 42: Specific PCR fragment of 345 bp in the B\*15:02:01-15:03:05, 15:05:01-15:06, 15:09-15:10:04, 15:13:01-15:13:02, 15:16:01-15:18:06, 15:21, 15:23, 15:25:01-15:25:03, 15:29, 15:31, 15:36-15:37, 15:39:01-15:40, 15:44, 15:48, 15:52, 15:55, 15:61-15:62, 15:64:01-15:64:02, 15:67, 15:69, 15:72, 15:74, 15:80, 15:88w, 15:89-15:91, 15:93, 15:95, 15:98, 15:103, 15:106-15:108, 15:112, 15:114-15:115, 15:119, 15:121, 15:123-15:124, 15:127, 15:131-15:134, 15:136, 15:138-15:139, 15:151, 15:153, 15:155-15:156, 15:158, 15:161-15:162, 15:170, 15:173, 15:176w, 15:177, 15:185-15:186, 15:188, 15:194-15:198, 15:200w, 15:204, 15:208, 15:210, 15:213-15:214, 15:216, 15:219-15:220, 15:222-15:223, 15:226N, 15:229-15:230, 15:235, 15:238, 15:240, 15:242-15:243, 15:250w, 15:252-15:255, 15:263, 15:265-15:266, 15:268, 15:271, 15:273-15:275, 15:281-15:283, 15:288-15:294N, 15:297, 15:301-15:302N, 15:306-15:308, 15:310-15:311, 15:312w, 15:313-15:314, 15:319, 15:323, 15:325, 15:328-15:330, 15:332, 15:335, 15:337-15:339, 15:341, 15:345, 15:354, 15:356-15:358 and 15:360-15:362 and the B\*46:06, 46:08, 46:13:01-46:13:03, 46:19, 46:21:01-46:21:02, 46:25-46:26, 46:33, 46:43 alleles. Specific PCR fragment of 460 bp in the B\*15:73 and 15:303 alleles. Specific PCR fragments of 345 and 460 bp in the B\*15:86 and 15:224 and the B\*46:11 and 46:18 alleles.

Primer mix 50: Specific PCR fragment of 85 bp in the B\*15:67 and 15:343 and the B\*35:110 and in the A\*68:56 alleles. Specific PCR fragment of 185 bp in the B\*15:206 allele.

Primer mix 51: Specific PCR fragment of 135 bp in the B\*15:98, 15:109 and 15:163 alleles. Specific PCR fragment of 175 bp in the B\*15:277 and 15:278 and the B\*35:122 and 56:29 alleles. Specific PCR fragment of 200 bp in the B\*15:246N allele.

Primer mix 52: Specific PCR fragment of 200 bp in the B\*15:104, 15:307 and 15:321Q and the B\*51:142 alleles. Specific PCR fragment of 345 bp in the B\*15:92 and B\*15:213 alleles.

Primer mix 54: Specific PCR fragment of 250 bp in the B\*15:187 and in the C\*12:36 alleles. Specific PCR fragment of 410 bp in the B\*15:97 allele.

Primer mix 55: Specific PCR fragment of 175 bp in the B\*15:214 and the B\*07:120, 18:81, 35:250, 40:150, 51:165 and 57:29 alleles. Specific PCR fragment of 210 bp in the B\*15:321Q allele. Specific PCR fragment of 255 bp in the B\*15:102 and 15:284 and the B\*51:30 and 73:01-73:02 alleles.

Primer mix 56: Specific PCR fragment of 70 bp in the B\*15:103 allele. Specific PCR fragment of 260 bp in the B\*15:227 and the B\*27:69, 37:23 and 40:213 alleles.

Primer mix 59: Specific PCR fragment of 230 bp in the B\*15:191 and 15:234 and in the C\*06:33 alleles. Specific PCR fragment of 375 bp in the B\*15:198 and the B\*35:161 alleles. Specific PCR fragment of 420 bp in the B\*15:94N and 15:294N alleles.

Primer mix 61: Specific PCR fragment of 160 bp in the B\*15:175 allele. Specific PCR fragment of 205 bp in the B\*15:125 and the B\*44:22, 44:105 and 49:24 alleles.

Primer mix 64: Specific PCR fragment of 90 bp in the B\*15:302N allele. Specific PCR fragment of 240 bp in the B\*15:301 allele. Specific PCR fragment of 365 bp in the B\*15:122 and in the C\*03:48 and C\*06:85 alleles.

Primer mix 65: Specific PCR fragment of 470 bp in the B\*15:232 and the B\*46:64 allele. Specific PCR fragment of 520 bp in the B\*15:129 and 15:342 allele.

Primer mix 67: Specific PCR fragment of 265 bp in the B\*15:53, 15:183, 15:212, 15:336 and 15:345 and the B\*46:10 and in the C\*16:85 alleles. Specific PCR fragment of 300 bp in the B\*15:132 allele.

Primer mix 68: Specific PCR fragment of 215 bp in the B\*15:134 allele. . Specific PCR fragment of 380 bp in the B\*15:247 and in the C\*05:03 alleles.

Primer mix 70: Specific PCR fragment of 90 bp in the B\*15:208 allele. Specific PCR fragment of 185 bp in the B\*15:138, 15:230, 15:241 and 15:297 and in the 07:68:01-07:68:03, 07:214, 07:237, 18:35, 18:110, 35:66, 40:77, 40:87:01-40:87:02, 40:121, 40:158, 40:222, 40:237, 44:150, 48:12, 48:14, 48:26 and 48:29 alleles.

Primer mix 71: Specific PCR fragment of 290 bp in the B\*15:53, 15:212, 15:336 and 15:347 and in the C\*16:85 alleles. Specific PCR fragment of 315 bp in the B\*15:205 allele. Specific PCR fragment of 400 bp in the B\*15:139 allele.

Primer mix 72: Specific PCR fragment of 110 bp in the B\*15:226N and the B\*35:216N and in the B\*39:87N alleles. Specific PCR fragment of 295 bp in the B\*15:140 and the B\*51:166 alleles. Specific PCR fragment of 390 bp in the B\*15:201 allele.

Primer mix 74: Specific PCR fragment of 75 bp in the B\*15:145 and 15:176 and the B\*35:237 and 52:28 alleles. Specific PCR fragment of 230 bp in the B\*15:262N allele.

Primer mix 75: Specific PCR fragment of 280 bp in the B\*15:146 allele. Specific PCR fragment of 390 bp in the B\*15:201 allele.

Primer mix 77: Specific PCR fragment of 185 bp in the B\*15:174 allele. Specific PCR fragment of 230 bp in the B\*15:123 and 15:220 alleles.

Primer mix 78: Specific PCR fragment of 110 bp in the B\*15:142 and the B\*07:29, 07:186, 08:01:11, 08:56:01, 08:133, 35:218, 35:256, 51:68, 51:176 and 57:49 and in the C\*07:02:30 alleles. Specific PCR fragment of 385 bp in the B\*15:147 and the B\*46:42 alleles. Specific PCR fragment of 470 bp in the B\*15:232 and the B\*46:64 allele.

Primer mix 79: Specific PCR fragment of 135 bp in the B15:270 alleles. Specific PCR fragment of 215 bp in the B\*15:182N allele. Specific PCR fragment of 420 bp in the B\*15:148 and 15:330 allele.

Primer mix 80: Specific PCR fragment of 85 bp in the B\*15:149N allele. Specific PCR fragment of 225 bp in the B\*15:263 allele. Specific PCR fragment of 340 bp in the B\*15:320 allele.

Primer mix 82: Specific PCR fragment of 80 bp in the B\*15:81 and the B\*53:35 and 58:47 alleles. Specific PCR fragment of 200 bp in the B\*15:246N allele. Specific PCR fragment of 245 bp in the B\*15:277 and the B\*35:222 and 40:76 and in the C\*05:30 and C\*06:125 alleles.

Primer mix 84: Specific PCR fragment of 155 bp in the B\*15:167 allele. Specific PCR fragment of 195 bp in the B\*15:211 and in the C\*03:168 alleles. Specific PCR fragment of 235 bp in the B\*15:197 and 15:215 and the B\*35:117 alleles.

Primer mix 85: Specific PCR fragment of 340 bp in the B\*15:157 allele. Specific PCR fragment of 395 bp in the B\*15:76, 15:101 and 15:255 and in the C\*07:335 and C\*14:62 alleles. Specific PCR fragment of 420 bp in the B\*15:164 allele.

Primer mix 86: Specific PCR fragment of 230 bp in the B\*15:166 allele. Specific PCR fragment of 260 bp in the B\*15:272N allele. Specific PCR fragment of 400 bp in the B\*15:193 allele.

Primer mix 87: Specific PCR fragment of 85 bp in the B\*15:71, 15:175, 15:204, 15:225 and 15:344 and the B\*18:29, 18:98, 35:50, 35:231, 35:280, 37:22, 40:248, 40:282, 41:12, 41:36, 46:14, 49:03, 51:151, 54:02, 55:16, 56:35, 57:39, 57:73 and 58:41 and in the C\*03:278 and C\*16:86 alleles. Specific PCR fragment of 295 bp in the B\*15:169 allele.

Primer mix 88: Specific PCR fragment of 240 bp in the B\*15:171 and in the C\*14:67 alleles. Specific PCR fragment of 450 bp in the B\*15:158 and 15:240 and in the C\*05:80 alleles.

Primer mix 89: Specific PCR fragment of 370 bp in the B\*15:160 and 15:282 and in the C\*03:84 and C\*07:275 alleles. Specific PCR fragment of 420 bp in the B\*15:94N and 15:294N alleles.

Primer mix 90: Specific PCR fragment of 95 bp in the B\*15:202 and 15:239 and the B\*13:58, 18:12, 35:10, 35:13, 35:16, 35:28, 35:69, 35:80, 35:226, 35:270, 37:01:01-37:01:05, 37:01:07-37:05, 37:07, 37:09-37:27, 37:29-37:56, 40:185, 40:209, 40:308, 44:83, 44:134, 49:03, 52:01:01:01-52:01:24, 52:01:27-52:01:28, 52:03-52:15, 52:17, 52:19-52:31:02, 52:33-52:45, 52:47-52:50, 53:17:01-53:17:02, 53:28, 53:38 and 78:05-78:06 alleles. Specific PCR fragment of 195 bp in the B\*15:173 and the B\*18:63, 39:90 and 50:11 alleles.

Primer mix 91: Specific PCR fragment of 135 bp in the B\*15:184 and 15:203 and 18:109 alleles. Specific PCR fragment of 325 bp in the B\*15:170 and 15:356 alleles.

Primer mix 92: Specific PCR fragment of 95 bp in the B\*15:266, 15:332 and the B\*13:37, 38:49 and 51:75 alleles. Specific PCR fragment of 155 bp in the B\*15:172 and 15:328 alleles. Primer mix 93: Specific PCR fragment of 255 bp in the B\*15:258N and 15:304N alleles. Specific PCR fragment of 295 bp in the B\*15:53, 15:177, 15:212, 15:336 and 15:347 and in the C\*16:85 allele. Specific PCR fragment of 385 bp in the B\*15:120 allele.

Primer mix 94: Specific PCR fragment of 260 bp in the B\*15:272N allele. Specific PCR fragment of 285 bp in the B\*15:123 allele. Specific PCR fragment of 325 bp in the B\*15:178 allele.

Primer mix 95: Specific PCR fragment of 160 bp in the B\*15:358 allele. Specific PCR fragment of 340 bp in the B\*15:181N allele. Specific PCR fragment of 395 bp in the B\*15:40, 15:47:01-15:47:02, 15:49, 15:52, 15:114, 15:117, 15:124, 15:138, 15:238 and 15:241 and the B\*46:06, 46:25 and 46:30 alleles.

**4**The following HLA-B\*15 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |  |  |
| --- | --- | --- | --- |
| **Alleles** | **Primer mix** | **Alleles** | **Primer mix** |
| B\*15:120, 15:258N | 93 | B\*15:206, 15:343 | 50 |
| B\*15:142, 15:147 | 78 | B\*15:208, 15:230 | 70 |
| B\*15:166, 15:193 | 86 | B\*15:301, 15:302N | 64 |
| B\*15:169, 15:225 | 87 |

The HLA-B\*15 subtyping kit cannot distinguish these silent mutations: the B\*15:01:01:01, 15:01:03, 15:01:03, 15:01:06-15:01:13, 15:01:15-15:01:16, 15:01:18-15:01:30 and 15:01:32-15:01:39 alleles, the B\*15:02:01-15:02:09 alleles, the B\*15:03:01-15:03:02 and 15:03:04-15:03:05 alleles, the B\*15:04:01-15:04:2 alleles, the B\*15:05:01-15:05:02 alleles, the B\*15:07:01-15:07:03 alleles, the B\*15:08:01-15:08:02 alleles, the B\*15:10:01 and 15:10:03-15:10:04 alleles, the B\*15:11:01-15:11:02 and 15:11:05-15:11:06 alleles, the B\*15:13:01-15:13:02 alleles, the B\*15:16:01-15:16:03 alleles, the B\*15:17:01:01-15:17:03 alleles, the B\*15:18:01 and 15:18:04-15:18:06 alleles, the B\*15:24:01-15:24:02 alleles, the B\*15:25:01-15:25:03 alleles, the B\*15:27:01-15:27:03 alleles, the B\*15:32:01-15:32:02 alleles, the B\*15:38:01-15:38:02 alleles, the B\*15:39:01-15:39:02 alleles, the B\*15:47:01-15:47:02 alleles,

the B\*15:64:01-15:64:02 alleles, the B\*15:78:01-15:78:03 or the B\*15:179:01-15:179:02 alleles.

5This lot of the B\*15 kit cannot distinguish the B\*15:159 and the B\*15:01:01:01, 15:01:01:03, 15:01:03, 15:01:06-15:01:13, 15:01:15-15:01:16, 15:01:18-15:01:30, 15:01:32-15:01:39, 15:92, 15:217, 15:231, 15:244, 15:251, 15:257, 15:261, 15:264, 15:267, 15:276, 15:279-15:280, 15:286, 15:296, 15:309, 15:315-15:318, 15:326, 15:333, 15:340, 15:346, 15:353, 15:355, 15:359 and 15:363 alleles or the B\*15:260 and the B\*15:82 and 15:256 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 66 may give rise to a lower yield of HLA-specific PCR product than the other HLA-B\*15 resolution primer mixes. A foot note has been added in the Specificity Table.

Changes in revision R02 compared to R01:

1. Primer mix 83 does not amplify the B\*15:159 and 15:260 and the B\*07:23, 18:95, 40:79, 40:190, 40:289, 41:14 and 46:52 alleles. Thus, this lot of the B\*15 kit cannot distinguish the B\*15:159 and the B\*15:01:01:01, 15:01:01:03, 15:01:03, 15:01:06-15:01:13, 15:01:15-15:01:16, 15:01:18-15:01:30, 15:01:32-15:01:39, 15:92, 15:217, 15:231, 15:244, 15:251, 15:257, 15:261, 15:264, 15:267, 15:276, 15:279-15:280, 15:286, 15:296, 15:309, 15:315-15:318, 15:326, 15:333, 15:340, 15:346, 15:353, 15:355, 15:359 and 15:363 alleles or the B\*15:260 and the B\*15:82 and 15:256 alleles. This has been corrected in the Specificity and Interpretation Tables.

Changes in revision R03 compared to R02:

1. Primer mix 77 may give rise to a lower yield of HLA-specific PCR product than the other HLA-B\*15 resolution primer mixes. A foot note has been added in the Specificity Table.

Changes in revision R04 compared to R03:

1. The expiry date has been corrected.

Changes in revision R05 compared to R04:

1. Primer mix 52 does not amplify the B\*15:98 or the C\*03:180 alleles, and this primer mix does amplify the B\*15:92 and 15:213 alleles. This has been corrected in the Specificity and Interpretation Tables.