Gel Documentation Form and Worksheet

HLA-B\*56 (101.571-06/06u) Lot No: 0F3 Expiry Date: 2019-09-01

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 7 and 18 may give rise to a lower yield of HLA-specific PCR product than the other B\*56 primer mixes.

Primer mixes 8, 10, 13, 17, 20 and 21 may have tendencies of unspecific amplifications.

Primer mixes 1, 9, 13 and 14 have a tendency to giving rise to primer oligomer formation.

Primer mix 6 may give rise to an unspecific amplification product of approx. 670 bp in some HLA-B alleles. This band should be disregarded in the interpretation of HLA-B\*56 typings.

Primer mix 24 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\*56 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 175 bp in the B\*56:40 allele. Specific PCR fragment of 285 bp in the B\*56:03 and the B\*40:10:02, 54:01:02 and 55:35 alleles.

Primer mix 6: Specific PCR fragment of 105 bp in the B\*56:05:01 and the B\*07:206, 08:26:03, 18:125, 27:05:18 and 39:63 alleles. Specific PCR fragment of 150 bp in the B\*56:32 and the B\*07:09w, 07:11w, 07:162w, 07:237w, 07:255w, 15:03:01:01-15:03:06, 15:16:01:01-15:16:03, 15:18:01:01-15:18:06, 15:23, 15:29, 15:39:01-15:40:02, 15:47:01w-15:47:02w, 15:49w, 15:61-15:62, 15:64:01-15:64:02, 15:65w, 15:67, 15:69, 15:72, 15:74, 15:80, 15:93, 15:95, 15:98, 15:103, 15:108, 15:115, 15:119, 15:127, 15:131-15:132, 15:134, 15:138, 15:153, 15:158, 15:161, 15:173, 15:176, 15:194-15:195, 15:197:01-15:198, 15:210, 15:220, 15:221w, 15:223, 15:226N, 15:238, 15:242:01-15:242:02, 15:254-15:255, 15:263, 15:266, 15:268, 15:274, 15:281-15:282, 15:288, 15:290, 15:292-15:294N, 15:306-15:307, 15:310-15:311, 15:314, 15:335, 15:337, 15:351, 15:354, 15:362, 15:364, 15:369, 15:376, 15:379w, 15:380N, 15:382, 15:388-15:389, 15:397, 15:402, 18:15, 18:21w, 18:30w, 18:57, 18:101, 35:11:01-35:11:02, 35:11:03w, 35:21, 35:58, 35:99w, 35:188, 35:232w, 35:277, 46:03w, 46:08, 46:62w, 48:25, 49:04:01-49:04:02, 51:37, 51:45, 53:06, 53:08:01-53:08:02, 53:28, 55:14, 55:68, 57:13w, 57:22, 58:06, 58:19 and 58:46 and in the C\*01:102w, C\*03:175, 03:199, 03:245, C\*08:113, C\*15:09, 15:19, 15:27, 15:61, 15:107 and 15:126 alleles.

Primer mix 7: Specific PCR fragment of 120 bp in the B\*56:05:01-56:06 and 56:21 and the B\*13:62, 27:153, 40:95, 49:18:01, 50:14, 51:01:01:01-51:01:03, 51:01:05-51:01:12, 51:01:14-51:02:03, 51:02:05-51:03, 51:05, 51:07:01-51:09:03, 51:11N-51:12, 51:13:02-51:14, 51:16-51:23, 51:24:03-51:24:05, 51:26-51:37, 51:39-51:41N, 51:43-51:44N, 51:48-51:55, 51:57-51:58, 51:60-51:61:02, 51:63:01, 51:65-51:80, 51:82-51:91, 51:92:02-51:134, 51:136-51:138, 51:140-51:147, 51:149N-51:156, 51:158:01-51:193, 51:195-51:196, 51:198, 51:200-51:212, 52:01:01:01-52:01:04, 52:01:06-52:01:14, 52:01:16-52:13, 52:15-52:24, 52:25:02-52:63, 55:01:04, 58:08:01-58:08:02 and 78:01:01-78:09 alleles. Specific PCR fragment of 170 bp in the B\*56:33 and the B\*14:54, 15:21, 15:44, 15:66, 15:189, 15:394, 15:404, 35:26, 35:82, 35:85, 35:135, 35:294, 38:28 and 59:08 alleles.

Primer mix 9: Specific PCR fragment of 145 bp in the B\*56:07 and the B\*07:149, 08:02, 08:117, 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, 44:40, 44:44, 44:130, 44:156, 44:210 and 59:08 alleles. Specific PCR fragment of 205 bp in the B\*56:31 and the B\*15:38:01-15:38:02, 15:185, 15:335, 15:364, 15:368, 18:01:01:01-18:01:13, 18:01:15-18:09, 18:12:01-18:15, 18:17N-18:19, 18:22-18:25, 18:27-18:34, 18:36-18:40, 18:42-18:55, 18:57, 18:59-18:60, 18:62-18:96, 18:98-18:100, 18:102-18:109, 18:111-18:121, 18:123, 18:125-18:130, 35:21, 35:24:01-35:24:02, 35:188, 35:190, 35:287, 39:43, 51:37, 51:45, 53:02, 53:06, 53:28, 57:14:01-57:14:02, 58:09 and 58:76 alleles.

Primer mix 12: Specific PCR fragment of 150 bp in the B\*56:25 and 56:37 and the B\*07:78, 13:16, 13:20, 13:31w, 13:48, 15:73w, 15:303w, 51:62, 51:106, 52:25:01-52:25:02, 54:33w, 55:09, 55:21w and 55:24 alleles. Specific PCR fragment of 180 bp in the B\*56:10 and 56:23 and the B\*13:06, 13:53, 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, 54:21-54:25, 54:27-54:32, 54:34-54:37, 55:02:01:01-55:02:10, 55:07, 55:10, 55:12, 55:16, 55:18-55:19, 55:21, 55:26, 55:30, 55:34-55:35, 55:37, 55:39, 55:42-55:43, 55:47-55:48, 55:50, 55:56-55:57, 55:61-55:63, 55:65, 55:67, 55:69-55:72, 55:77, 55:80, 59:01:01:01-59:01:01:02 and 59:05-59:08 alleles. Specific PCR fragment of 150 and 180 bp in the B\*15:42, 54:20, 55:01:01-55:01:16, 55:03, 55:05, 55:11, 55:15, 55:21, 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, 55:73-55:75, 55:78 and in the C\*01:90 and C\*15:128 alleles.

Primer mix 13: Specific PCR fragment of 70 bp in the B\*56:30 and the B\*44:174 alleles. Specific PCR fragment of 180 bp in the B\*56:11 and 56:22 and the B\*15:58, 15:133, 15:229, 15:253, 35:03:01:01-35:03:05, 35:03:07-35:03:21, 35:06, 35:13, 35:36, 35:38, 35:55-35:56, 35:59:01-35:59:02, 35:70, 35:74-35:75, 35:84-35:85, 35:98, 35:106, 35:109, 35:127-35:128, 35:136, 35:150:01-35:153, 35:155-35:156, 35:160, 35:163, 35:167, 35:169, 35:179, 35:181, 35:193, 35:195, 35:198, 35:204-35:205, 35:221, 35:223, 35:231, 35:235-35:237, 35:242-35:243, 35:246, 35:256-35:257, 35:261, 35:267, 35:274, 35:278-35:279, 35:281-35:282, 35:290, 35:296, 35:298, 35:312, 35:320, 35:326, 35:329, 37:48, 38:30, 38:65, 39:32-39:33, 39:74, 44:251, 46:61, 51:13:01-51:13:02, 51:92:01-51:92:02, 51:172, 52:14, 53:04, 53:31, 53:33, 57:76 and 58:34 and in the C\*08:138 alleles.

Primer mix 15: Specific PCR fragment of 160 bp in the B\*56:13 and the B\*44:15, 44:18, 44:140, 45:01:01-45:01:02, 45:04-45:08, 45:10-45:19, 55:56 and 82:01-82:03 alleles. Specific PCR fragment of 385 bp in the B\*56:08, 56:14 and 56:28N and the B\*35:186 alleles.

Primer mix 17: Specific PCR fragment of 80 bp in the B\*56:38N allele. Specific PCR fragment of 180 bp in the B\*56:05:01-56:06, 56:15 and 56:21 and the B\*07:20, 07:24, 07:60, 07:100, 07:131, 07:220, 07:223, 08:21, 08:25, 13:62, 13:86, 15:09-15:10:04, 15:30, 15:37, 15:45, 15:48, 15:63, 15:90, 15:99, 15:150, 15:243, 15:248, 15:287, 15:312-15:313, 15:324, 15:329, 15:338, 15:361, 27:76, 27:153, 35:02:01-35:02:08, 35:04:01-35:04:03, 35:09:01-35:09:03, 35:12:01-35:12:03, 35:18, 35:22, 35:34, 35:39, 35:44, 35:81, 35:83, 35:95, 35:129N, 35:149, 35:154, 35:162, 35:172, 35:182-35:184, 35:199, 35:201, 35:211, 35:220, 35:233, 35:251, 35:258, 35:266, 35:270, 35:273, 35:285, 35:292, 35:309, 35:311, 35:316, 35:321, 35:323, 40:05, 40:26, 40:28, 40:63, 40:92, 40:174, 40:324, 40:343, 41:17, 44:62, 44:77, 44:82, 44:107, 44:123, 44:184, 44:213, 49:18:01-49:18:02, 50:14, 51:01:01:01-51:01:14, 51:01:16-51:01:31, 51:01:33-51:01:38, 51:01:40-51:09:03, 51:11N-51:12, 51:18-51:20, 51:22-51:24:05, 51:26-51:30, 51:32-51:33, 51:35, 51:38-51:41N, 51:43-51:44N, 51:46, 51:48-51:61:02, 51:64-51:80, 51:83-51:84, 51:86-51:91, 51:94-51:96, 51:98N-51:100, 51:102-51:105, 51:107-51:142, 51:145-51:152, 51:154-51:156, 51:158:01-51:171, 51:173Q-51:181, 51:183-51:191, 51:193-51:196, 51:198-51:213, 52:01:01:01-52:01:20, 52:01:22-52:07, 52:09-52:13, 52:15-52:24, 52:27-52:36, 52:38, 52:40-52:61, 53:19, 53:36, 53:42, 55:08, 55:51, 57:02:01-57:03:02, 57:05, 57:07, 57:09, 57:12, 57:17, 57:28N, 57:39, 57:42, 57:46, 57:57, 57:63, 57:66, 57:70, 57:80, 57:82, 57:84, 58:08:01-58:08:02, 58:28:01-58:28:02, 58:65, 78:01:01-78:06 and 78:09 and in the C\*03:81, 03:152 and 03:317 alleles.

Primer mix 18: Specific PCR fragment of 295 bp in the B\*56:16 and the B\*40:149, 41:22, 49:25, 54:21, 55:10, 57:58, 57:80, 67:06?-67:07? and 82:01-82:03 alleles. Specific PCR fragment of 405 bp in the B\*56:28N allele.

Primer mix 19: Specific PCR fragment of 75 bp in the B\*56:29 and the B\*07:176 alleles. Specific PCR fragment of 105 bp in the B\*56:08 and 56:14 and in the C\*03:342, C\*07:46 and 07:356 alleles. Specific PCR fragment of 185 bp in the B\*56:19N and the B\*39:40:01N-39:40:02N alleles.

Primer mix 21: Specific PCR fragment of 115 bp in the B\*56:17, 56:23, 56:42 and 56:44 and the B\*07:16, 07:37, 07:50, 07:75, 07:91, 07:207, 07:228, 08:23, 08:54:01-08:54:02, 08:110, 14:14, 14:18, 15:03:01:01-15:03:06, 15:09-15:10:04, 15:18:01:01-15:18:06, 15:29, 15:37, 15:47:01-15:47:02, 15:49, 15:51-15:52, 15:54, 15:62, 15:64:01-15:64:02, 15:68-15:69, 15:72, 15:74, 15:80, 15:90-15:91, 15:93, 15:98-15:99, 15:103, 15:108, 15:114, 15:123-15:124, 15:127, 15:131-15:134, 15:151, 15:153, 15:156, 15:158, 15:161, 15:173, 15:176, 15:186, 15:197:01-15:198, 15:200, 15:210, 15:220-15:221, 15:226N, 15:229, 15:235, 15:238, 15:242:01-15:243, 15:252-15:253, 15:259, 15:263, 15:266, 15:274-15:275:02, 15:281-15:282, 15:290, 15:292-15:293, 15:306-15:307, 15:311-15:314, 15:323, 15:329, 15:335, 15:337-15:338, 15:351, 15:354, 15:369, 15:379-15:380N, 15:382, 15:388-15:389, 18:37, 18:125, 27:18, 27:23, 27:44, 27:77, 27:129, 27:131, 35:25-35:26, 35:49, 39:05:01-39:05:02, 39:07-39:08, 39:11, 39:13:01-39:13:02, 39:20, 39:37, 39:49, 39:55-39:56, 39:113, 39:118, 40:12, 40:46, 40:93, 42:11, 48:01:01-48:40, 55:18, 55:31, 55:78 and 81:08 alleles. Specific PCR fragment of 170 bp in the B\*56:26 allele.

Primer mix 22: Specific PCR fragment of 105 bp in the B\*56:06 and 56:45 and the B\*07:65, 07:134 and 35:76 alleles. Specific PCR fragment of 175 bp in the B\*56:18, 56:31-56:32 and 56:36 and the B\*07:177, 08:37, 08:89, 08:107, 15:50, 15:69, 15:93, 15:186, 15:199, 15:348, 15:368, 18:01:01:01-18:01:13, 18:01:15-18:01:17, 18:01:19-18:09, 18:11-18:15, 18:17N-18:19, 18:22-18:25, 18:27-18:34, 18:37-18:40, 18:42-18:49, 18:51-18:55, 18:59-18:60, 18:62-18:85, 18:87-18:96, 18:98-18:100, 18:102-18:105, 18:107-18:121, 18:123, 18:125-18:130, 35:35, 35:93, 35:188, 35:190, 35:262, 35:277, 37:35, 37:56, 38:52, 39:07, 39:43, 39:91, 40:166, 42:09, 44:90, 45:06, 46:03, 46:21:01-46:21:02, 46:29, 54:09, 54:14, 55:14, 55:23, 55:68, 57:26, 58:05, 58:35 and 58:76 and in the C\*01:102, C\*06:89, C\*07:123, 07:173, 07:294, C\*08:113, C\*12:113, C\*15:09, 15:19, 15:27, 15:61, 15:107, 15:126 and C\*16:20 alleles.

Primer mix 23: Specific PCR fragment of 85 bp in the B\*56:20:01-56:20:02 and the B\*07:78, 40:48 and 49:40 and in the C\*01:90, C\*07:536, C\*15:91 and 15:128 alleles. Specific PCR fragment of 350 bp in the B\*56:24 allele.

**4**The HLA-B\*56 primer set cannot separate the B\*56:09 and the B\*35:78 alleles. These alleles can be distinguished by the HLA-B low resolution and/or B\*35 kits.

The HLA-B\*56 primer set cannot separate the B\*56:23 and the B\*55:31 and B\*55:78 alleles. These alleles can be distinguished by the HLA-B low resolution and/or B\*55 kits.

The HLA-B\*56 primer set cannot separate the B\*56:50 and the B\*55:13, 55:27-55:28, 55:32 and 55:58 alleles. These alleles can be distinguished by the HLA-B low resolution and/or B\*55 kits.

**5**The following HLA-B\*56 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |
| --- | --- |
| **Alleles** | **Primer mix** |
| B\*56:17, 56:26 | 21 |
| B\*56:18, B\*07:65 | 22 |
| B\*56:19N, 56:29 | 19 |

The HLA-B\*56 subtyping kit cannot distinguish the silent mutations in the B\*56:01:01:01-56:01:08 alleles, the B\*56:02:01-56:02:02 and the B\*56:20:01-56:20:02 alleles.

‘w’, may be weakly amplified.

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