Gel Documentation Form and Worksheet

HLA-B\*46 (101.544-06/06u) Lot No: 8E3 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**

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| --- |
| 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, 10, 13, 14 and 16 may have tendencies of unspecific amplifications.

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\*49 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 4: Specific PCR fragment of 130 bp in the B\*46:04 and 46:40:01-46:40:02 and the B\*18:06, 35:74 and 35:186 alleles. Specific PCR fragment of 245 bp in the B\*46:14 and the B\*18:06, 40:73 and 73:01w-73:02w and in the C\*03:278 and C\*16:86 alleles.

Primer mix 6: Specific PCR fragment of 120 bp in the B\*46:20 and the B\*55:19 alleles. Specific PCR fragment of 215 bp B\*46:06 and 46:41N and the B\*07:09, 07:11, 07:17, 07:162, 07:237, 07:255, 15:138, 15:241, 15:297, 18:35, 35:66, 40:38, 40:52, 40:59-40:60, 40:158, 40:222, 48:14 and 48:23 and in the C\*07:38:01-07:38:02 alleles.

Primer mix 7: Specific PCR fragment of 105 bp in the B\*46:16 allele. Specific PCR fragment of 140 bp in the B\*46:07N and the B\*14:07N, 39:40:01N-39:40:02N and 56:19N alleles. Specific PCR fragment of 180 bp in the B\*46:56 allele.

Primer mix 8: Specific PCR fragment of 190 bp in the B\*46:39 and the B\*35:298 alleles. Specific PCR fragment of 380 bp in the B\*46:06, 46:08, 46:11, 46:13:01-46:13:03, 46:18-46:19, 46:21:01-46:21:02, 46:25, 46:33 and 46:43 and the B\*15:02:01-15:03:06, 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, 15:39:01-15:40:02, 15:42, 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, 15:86, 15:88-15:91, 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, 15:168, 15:170, 15:173, 15:176-15:177, 15:185-15:186, 15:188, 15:194-15:198, 15:200, 15:204, 15:208, 15:210, 15:213-15:214, 15:216, 15:219-15:220, 15:222-15:224, 15:226N, 15:229-15:230, 15:235, 15:238, 15:240, 15:242:01-15:243, 15:249-15:250, 15:252-15:255, 15:263, 15:265-15:266, 15:268, 15:271, 15:273-15:275:02, 15:281-15:283, 15:288-15:294N, 15:297, 15:301-15:302N, 15:306-15:308, 15:310-15:314, 15:319, 15:323, 15:325, 15:328-15:330, 15:332, 15:335, 15:337-15:339, 15:341, 15:345, 15:348, 15:351, 15:354, 15:356-15:358, 15:360-15:361, 15:364, 15:366, 15:369, 15:376, 15:378, 15:380, 15:382-15:384, 15:388-15:389, 15:393-15:397, 15:399 and 15:402-15:404 alleles.

Primer mix 10: Specific PCR fragment of 100 bp in the B\*46:05 and the B\*07:204, 07:210, 14:02:13, 15:125, 35:54, 51:140 and in the C\*05:01:28, C\*07:02:30 and C\*08:16:02 alleles. Specific PCR fragment of 160 bp in the B\*46:38 alleles. Specific PCR fragment of 315 bp in the B\*46:15N allele.

Primer mix 12: Specific fragment of 100 bp in the B\*46:40:01-46:40:02 and the B\*35:186 alleles. Specific PCR fragment of 170 bp in the B\*46:10 allele. Specific PCR fragment of 305 bp in the B\*46:55N allele. Specific PCR fragment of 380 bp in the B\*46:19 and the B\*15:48, 15:108, 15:136, 15:235 and 15:249 alleles.

Primer mix 13: Specific PCR fragment of 105 bp in the B\*46:11 and 46:18 and the B\*07:78, 13:02:01-13:02:14, 13:02:16-13:03, 13:08-13:09, 13:14-13:16, 13:18-13:19, 13:27, 13:30, 13:32-13:34, 13:37-13:38, 13:40, 13:42, 13:44-13:45, 13:48-13:49N, 13:53-13:56:02N, 13:58, 13:65-13:70, 13:74-13:75, 13:81-13:82, 13:84-13:85, 13:88-13:89, 13:91, 13:93-13:94, 13:96, 14:37, 15:42, 35:60, 44:15, 44:18, 45:01:01-45:01:02, 45:03-45:08, 45:10-45:19, 49:01:01-49:03, 49:06-49:14, 49:16-49:17, 49:19N-49:46, 50:01:01-50:02, 50:04-50:08, 50:10-50:11, 50:13, 50:15, 50:18-50:19, 50:31-50:32, 50:34-50:40, 50:43-50:46, 50:48-50:49, 51:15, 51:157, 52:25:01, 54:01:01-54:03, 54:05N, 54:07-54:08N, 54:10, 54:12-54:13, 54:16-54:30, 54:32, 54:34-54:37, 55:01:01-55:01:03, 55:01:05-55:01:06, 55:01:08-55:03, 55:05, 55:07, 55:09-55:12, 55:15-55:16, 55:18-55:19, 55:22, 55:24-55:26, 55:29-55:31, 55:33-55:38, 55:40-55:41, 55:43, 55:45-55:48, 55:50, 55:52, 55:54-55:57, 55:59-55:60, 55:62-55:67, 55:69-55:78, 55:80, 56:01:01:01-56:01:08, 56:07-56:08, 56:13-56:14, 56:16-56:17, 56:19N-56:20:02, 56:23-56:30, 56:33-56:42, 56:44-56:49, 59:01:01:01-59:01:01:02 and 59:04-59:09 alleles. Specific PCR fragment of 220 bp in the B\*46:22 and 46:32 and the B\*15:04:01:01-15:04:04, 15:137, 35:265, 51:61:01-51:61:02 and 52:21 and in the C\*12:183 alleles. Specific PCR fragment of 105 bp and 220 bp in the B\*13:31, 13:41, 15:303, 54:33, 55:21 and 56:43 alleles.

Primer mix 15: Specific PCR fragment of 305 bp in the B\*46:55N allele. Specific PCR fragment of 330 bp in the B\*46:01:03, 46:01:16, 46:11, 46:13:01-46:13:02, 46:18, 46:21:02 and 46:33 and the B\*15:02:09, 15:03:02, 15:03:04, 15:05:02, 15:07:03, 15:13:02, 15:18:06, 15:27:03, 15:38:02, 15:39:02, 15:42, 15:48, 15:86, 15:179:01, 15:188, 15:224, 15:269, 15:275:01, 15:383 and 15:393 alleles.

Primer mix 18: Specific PCR fragment of 95 bp in the B\*46:23 allele. Specific PCR fragment of 265 bp in the B\*46:57 allele.

Primer mix 19: Specific PCR fragment of 160 bp in the B\*46:38 allele. Specific PCR fragment of 230 bp in the B\*46:24 and the B\*15:19 and 15:304N alleles.

Primer mix 20: Specific PCR fragment of 50 bp in the B\*46:28 and the B\*15:340 and 57:78 alleles. Specific PCR fragment of 225 bp in the 46:34 allele.

Primer mix 21: Specific PCR fragment of 235 bp in the B\*46:51Q allele. Specific PCR fragment of 270 bp in the B\*46:26 allele.

Primer mix 22: Specific PCR fragment of 180 bp in the B\*46:56 allele. Specific PCR fragment of 215 bp in the B\*46:27 and the B\*15:292, 15:401, 35:113, 44:98 and 52:31:01-52:31:02 and in the A\*02:376, C\*03:125 and 03:176 alleles.

**4**The HLA-B\*46 primer set cannot separate the B\*46:31, 46:47 and 46:49 from the B\*15:318 allele. These alleles can be distinguished by the HLA-B low resolution kit and/or the HLA-B\*15 kit.

**5**The following HLA-B\*46 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |
| --- | --- |
| **Alleles** | **Primer mix** |
| B\*46:23, 46:57 | 18 |
| B\*46:28, 46:34 | 20 |

The HLA-B\*46 subtyping kit cannot distinguish the silent mutations in the B\*46:01:01-46:01:02, 46:01:04-46:01:15 and 46:01:17-46:01:22 alleles, the B\*46:01:03 and 46:01:16 alleles, the B\*46:13:01-46:13:02 alleles or the B\*46:40:01-46:40:02 alleles.

‘w’, might be weakly amplified.