Gel Documentation Form and Worksheet

HLA-C\*16 (101.627-12/12u) Lot No: 7E1 Expiry Date: 2019-06-01

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 2 and 3 may give rise to a lower yield of HLA-specific PCR product than the other C\*16 primer mixes.

Primer mixes 9, 14 and 23 may have tendencies of unspecific amplifications.

Primer mix 22 has a tendency to giving rise to primer oligomer formation.

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.

Change in revision R01 compared to R00:

1. Primer mix 21 does not amplify the C\*16:63 allele. Thus, this lot of the C\*16 subtyping kit cannot distinguish the C\*16:63 and C\*16:02:01-16:02:13, 16:47, 16:57, 16:60, 16:69, 16:74 and 16:84 alleles. This has been corrected in the Specificity and Interpretation Tables.









**1**HLA-C\*16 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 6: Specific PCR fragment of 125 bp in the C\*16:11 and 16:39:01-16:39:02 and the C\*02:21 alleles. Specific PCR fragment of 160 bp in the C\*16:10 and in the A\*24:106 alleles. Specific PCR fragment of 210 bp in the C\*16:06 and the C\*07:216 alleles.

Primer mix 7: Specific PCR fragment of 100 bp in the C\*16:09 and the C\*02:34 alleles. Specific PCR fragment of 170 bp in the C\*16:45 and the C\*04:14, 04:68 and 05:112 and in the A\*24:96 and A\*24:146 alleles. Specific PCR fragment of 210 bp in the C\*16:07:01 allele.

Primer mix 8: Specific PCR fragment of 130 bp in the C\*16:08 and 16:38 and the C\*08:96 and 15:114 alleles. Specific PCR fragment of 160 bp in the C\*16:89N allele. Specific PCR fragment of 435 bp in the C\*16:53, 16:68 and 16:88 alleles.

Primer mix 9: Specific PCR fragment of 85 bp in the C\*16:12 and the C\*02:79 and 04:69 alleles. Specific PCR fragment of 140 bp in the C\*16:52 and the C\*06:133 alleles. Specific PCR fragment of 205 bp in the C\*16:35 and 16:48 and the C\*06:118, 07:31:01-07:31:02, 07:177, 07:514 and 14:15 alleles.

Primer mix 10: Specific PCR fragment of 215 bp in the C\*16:19 and the C\*04:101, 05:117, 07:114 and 08:135 alleles. Specific PCR fragment of 350 bp in the C\*16:13 and 16:61 and the C\*05:81, 06:87, 07:24, 07:218, 12:45, 12:166 and 14:65 alleles.

Primer mix 11: Specific PCR fragment of 170 bp in the C\*16:20 and the C\*15:75 and in the A\*24:73, A\*24:157, B\*07:66 and B\*51:55 alleles. Specific PCR fragment of 540 bp in the C\*16:15:01-16:15:02, 16:25 and 16:64 and the C\*04:14, 04:68, 07:53 and 07:216 alleles.

Primer mix 12: Specific PCR fragment of 100 bp in the C\*16:17 and 16:67 and the C\*01:27 alleles. Specific PCR fragment of 210 bp in the C\*16:22 allele. Specific PCR fragment of 245 bp in the C\*16:16Q allele.

Primer mix 13: Specific PCR fragment of 130 bp in the C\*16:14 and the C\*06:32 and 12:40 alleles. Specific PCR fragment of 240 bp in the C\*16:77N and the C\*06:171N, 07:164N and 07:451N alleles.

Primer mix 15: Specific PCR fragment of 85 bp in the C\*16:04:03 allele. Specific PCR fragment of 120 bp in the C\*16:39:01-16:39:02 alleles. Specific PCR fragment of 145 bp in the C\*16:21 and 16:80 and the C\*02:14:01-02:14:02, 02:107, 04:42:01-04:42:02, 04:220, 05:43, 06:05, 07:02:09, 08:37, 12:16, 12:147, 15:23 and 15:63 alleles.

Primer mix 16: Specific PCR fragment of 165 bp in the C\*16:90 allele. Specific PCR fragment of 375 bp in the C\*16:06-16:07:02 and the C\*01:05, 01:21, 01:36, 01:55, 01:79:01-01:79:02, 01:120, 02:02:01-02:02:03, 02:02:05-02:02:08, 02:02:10-02:04, 02:06:01-02:16:02, 02:18-02:36, 02:38N-02:40:02, 02:42-02:56, 02:58-02:61, 02:63-02:73, 02:75-02:80, 02:82, 02:84-02:120, 03:05, 03:13:01-03:13:02, 03:25, 03:27, 03:35, 03:135, 03:167, 03:178, 03:198, 03:267, 03:292, 03:296, 04:01:01:01-04:01:23, 04:01:25-04:01:75, 04:03:01-04:20, 04:23-04:36, 04:38-04:39, 04:41-04:79, 04:81-04:99, 04:101-04:109, 04:111-04:116, 04:118-04:177, 04:179-04:223, 04:225N-04:229, 04:231, 04:233N-04:241, 04:243-04:244, 05:01:01:01-05:01:20, 05:01:22-05:01:32, 05:03-05:06, 05:08-05:09:03, 05:11-05:15, 05:17-05:30, 05:32-05:84, 05:86-05:95, 05:97-05:103:02, 05:105-05:106:02, 05:108-05:135, 06:101, 06:127:01:01-06:127:02, 06:136, 06:144, 07:01:01:01-07:01:10, 07:01:12-07:01:27, 07:01:29-07:03, 07:05-07:09, 07:13-07:30, 07:32N-07:33N, 07:35-07:42, 07:44, 07:46-07:62, 07:64-07:100, 07:102-07:138, 07:140-07:141:02, 07:143-07:176, 07:178-07:180, 07:182-07:183, 07:185-07:194, 07:197-07:271, 07:273-07:294, 07:296-07:301, 07:303-07:322, 07:325-07:327, 07:330:01-07:331, 07:333-07:335, 07:337, 07:339-07:345, 07:347N-07:353, 07:356, 07:359-07:360, 07:362-07:363, 07:368:01-07:377, 07:379-07:393N, 07:396-07:402, 07:404-07:405, 07:407-07:419, 07:421-07:425, 07:427, 07:429-07:443, 07:445-07:446, 07:448-07:458, 07:460-07:462, 07:464-07:465, 07:468-07:479, 07:481-07:484N, 07:486, 07:488-07:500, 07:502-07:510, 07:512-07:513Q, 07:515-07:522, 07:524-07:530, 08:01:01-08:01:10, 08:01:12-08:11, 08:13-08:23, 08:25-08:33:03, 08:35-08:43, 08:45-08:60, 08:62-08:63, 08:65-08:81, 08:83-08:125, 08:127N-08:136, 12:02:01-12:02:11, 12:02:13-12:02:14, 12:08, 12:10:01-12:10:02, 12:14:01-12:14:02, 12:16-12:18:02, 12:21-12:22, 12:27, 12:30, 12:36, 12:40-12:41, 12:49, 12:56, 12:64, 12:67-12:69, 12:72-12:74, 12:80N, 12:83-12:86, 12:96, 12:103-12:106, 12:112, 12:114, 12:117-12:118, 12:123-12:124, 12:126-12:128, 12:130, 12:132, 12:136-12:137, 12:142, 12:145-12:146, 12:148N, 12:151, 12:155Q, 12:161-12:162, 12:164, 12:166, 12:168-12:169, 12:177, 12:179, 12:181, 14:09, 14:28:02, 15:22, 15:65, 15:72, 17:16 and 18:01-18:10 alleles. Specific PCR fragment of 165 bp and 375 bp in the C\*02:83 and 08:24 alleles.

Primer mix 17: Specific PCR fragment of 180 bp in the C\*16:26, 16:46, 16:55 and 16:64 and the C\*02:49, 02:75, 02:115, 04:01:01:01-04:01:09, 04:01:11-04:01:22, 04:01:24-04:01:73, 04:01:74w, 04:01:75, 04:03:01-04:10, 04:12-04:20, 04:23-04:26, 04:28-04:32, 04:34-04:51, 04:53-04:54, 04:56-04:106, 04:108-04:115N, 04:117-04:129, 04:131-04:168, 04:170N-04:171, 04:173N-04:230, 04:232-04:244, 05:25, 05:42, 06:05, 06:76:02, 07:02:09, 08:28, 12:28, 12:132, 12:135, 12:146, 15:25 and 15:62 allele. Specific PCR fragment of 235 bp in the C\*16:97 allele.

Primer mix 18: Specific PCR fragment of 120 bp in the C\*16:38 and the C\*08:96 and 15:114 alleles. Specific PCR fragment of 255 bp in the C\*16:26, 16:46, 16:55 and 16:64 and the C\*01:23, 01:58, 02:49, 02:75, 02:115, 04:01:72, 04:03:01-04:03:03, 04:06, 04:42:02, 04:80, 04:140, 04:147, 04:160, 04:171, 04:220, 05:25, 05:42, 06:02:01:01-06:02:01:03, 06:02:03-06:02:09, 06:02:11-06:25, 06:27-06:29, 06:31-06:52, 06:54-06:124, 06:126-06:131, 06:133-06:168, 06:170-06:187, 07:01:01:01-07:01:22, 07:01:24-07:02:10, 07:02:12-07:02:76, 07:04:01:01-07:04:04, 07:04:06-07:06, 07:08-07:15, 07:17:01-07:19, 07:21-07:33N, 07:35, 07:37-07:50, 07:52-07:55N, 07:57-07:58, 07:61N-07:63, 07:65-07:78:02, 07:80-07:87, 07:89-07:95, 07:96:02-07:108:02, 07:110-07:126, 07:128-07:172:01, 07:173-07:176, 07:178-07:180, 07:182-07:226, 07:228-07:262, 07:264N-07:294, 07:296-07:326, 07:329N-07:354, 07:356-07:366, 07:368:01-07:377, 07:379-07:389, 07:391-07:401, 07:403-07:437N, 07:439-07:530, 08:28, 12:28, 12:132, 12:135, 12:146, 15:25, 15:62, 17:11 and 18:01-18:10 alleles.

Primer mix 19: Specific PCR fragment of 100 bp in the C\*16:28 and 16:67 and the C\*01:180 and 06:90 alleles. Specific PCR fragment of 240 bp in the C\*16:29, 16:31 and 16:50 and the C\*01:10, 02:05:01-02:05:03, 02:17, 06:08, 06:22, 12:119, 14:25 and 17:21 and in the B\*07:239, B\*14:46 and B\*40:243 alleles.

Primer mix 20: Specific PCR fragment of 95 bp in the C\*16:27 and 05:112 alleles. Specific PCR fragment of 145 bp in the C\*16:32 allele. Specific PCR fragment of 210 bp in the C\*16:23 allele.

Primer mix 21: Specific PCR fragment of 445 bp in the C\*16:40 and 16:53 alleles. Specific PCR fragment of 595 bp in the C\*16:49 allele.

Primer mix 22: Specific PCR fragment of 85 bp in the C\*16:58 allele. Specific PCR fragment of 210 bp in the C\*16:24 and the 03:108, 03:150, 07:25 and 07:404 alleles.

Primer mix 23: Specific PCR fragment of 95 bp in the C\*16:42 and 16:56 and the C\*05:56, 08:69 and 12:131 alleles. Specific PCR fragment of 170 bp in the C\*16:30N allele.

**4**The HLA-C\*16 primer set cannot separate the C\*16:04:01, 16:33, 16:66, 16:78, 16:82 and the C\*12:176 alleles. These alleles can be distinguished by the HLA-C low resolution kit and/or the HLA-C\*12 high resolution kit.

**5**The following HLA-C\*16 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |  |  |
| --- | --- | --- | --- |
| **Alleles** | **Primer mix** | **Alleles** | **Primer mix** |
| C\*16:15:01-16:15:02, C\*16:20 | 11 | C\*16:27, C\*16:32 | 20 |
| C\*16:16Q, C\*16:17 | 12 | C\*16:28, C\*16:31, 16:50 | 19 |
| C\*16:24, C\*16:58 | 22 | C\*16:30N, C\*16:56 | 23 |

**6**This lot of the C\*16 subtyping kit cannot distinguish the C\*16:63 and C\*16:02:01-16:02:13, 16:47, 16:57, 16:60, 16:69, 16:74 and 16:84 alleles.

The HLA-C\*16 subtyping kit cannot distinguish the following silent mutations: the C\*16:01:01:01-16:01:01:02, 16:01:03-16:01:15 and 16:01:17-16:01:22 alleles, the C\*16:01:02 and 16:01:16 alleles, the C\*16:02:01-16:02:13 alleles, the C\*16:15:01-16:15:02 alleles or the C\*16:39:01 and 16:39:02 alleles.

‘w’ might be weakly amplified.