Gel Documentation Form and Worksheet

HLA-C\*04 (101.612-12/12u) Lot No: 0F2 Expiry Date: 2019-11-01

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 7, 24, 31, 36, 38 and 41 have a tendency to giving rise to primer oligomer formation.

Primer mixes 5, 11, 15, 19, 26, 43 and 47 may have tendencies of unspecific amplification, most pronounced in primer mix 15.

Primer mix 16 may give rise to a lower yield of HLA-specific PCR product than the other C\*04 primer mixes.

Primer mix 28 may give rise to a long unspecific amplification product of approximately 500 bp. This should be disregarded when interpreting the C\*04 typings.

In primer mix 27 the positive control band may be weaker than for other HLA-C\*04 primer mixes.

Primer mix 64 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.

Changes in revision R01 compared to R00:

1. Primer mix 51 does not amplify the C\*04:165 allele. This has been corrected in the Specificity and Interpretation Tables.

Change in revision R02 compared to R02:

1. Primer mix 16 does not amplify the C\*04:15:02 and 04:17 and the C\*03:13:01, 03:35:01, 03:198, 08:01:07, 08:02:07 and 08:33:02 alleles. Thus, this lot of the C\*04 subtyping kit cannot distinguish the C\*04:15:02 and the C\*04:224 and 04:230 alleles, or the C\*04:17 and C\*04:100 alleles. This has been corrected in the Specificity and Interpretation Tables.

















**1**HLA-C\*04 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 3.26.0, 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**The following HLA-C\*04 primer mixes have two or more product sizes:

|  |  |  |  |
| --- | --- | --- | --- |
| Primer Mix | Size of spec. PCR product | Amplified HLA-C\*04 alleles3 | Other amplified HLA Class I alleles4 |
|  **6** | 95 bp 215 bp | \*04:05\*04:112, 04:169 | \*14:73, 15:36 |
|  **9** | 110 bp180 bp 220 bp | \*04:35\*04:30\*04:09N |  |
| **10** | 190 bp 220 bp | \*04:31, 04:91\*04:10-04:11, 04:36, 04:55, 04:153, 04:169, 04:210, 04:214-04:215N | \*03:231, 05:78:01-05:78:02, 15:36 |
| **12** | 125 bp 165 bp | \*04:11, 04:29, 04:36, 04:55, 04:172, 04:214w\*04:11, 04:33, 04:169, 04:172  | \*03:231, 03:248, 07:125, 07:356, 07:531, 12:194, 16:62, **B\*07:267****\***02:104, 03:248, 05:141, 12:194, 15:100, 16:62, **B\*07:267** |
| **13** | 120 bp215 bp270 bp | \*04:255N\*04:12, 04:132\*04:52, 04:55  | \*03:231 |
| **14** | 155 bp 185 bp | \*04:16, 04:163, 04:223:01-04:223:02\*04:18 | \*02:104, 03:248, 05:64:01-05:64:02, 08:19, 15:100, **A\*24:52** |
| **16** | 85 bp 130 bp | \*04:123N\*04:15:01, 04:15:03, 04:37  | \*18:07N\*03:05, 03:13:02, 03:25, 03:27, 03:35:02, 03:135, 03:167, 03:296, 03:302, 08:01:07, 14:09, 14:45, **B\*15:78:03** |
| **17** | 245 bp320 bp | \*04:234N\*04:17, 04:80, 04:100 | \*01:50, 14:54 |
| **18** | 125 bp 220 bp | \*04:70\*04:19, 04:94:01-04:94:02 | \*06:101, 12:10:01-12:10:02, 18:03, **B\*15:27:01-15:27:03, B\*15:109, B\*15:327, B\*15:344, B\*15:398** |
| **19** | 120 bp 150 bp430 bp | \*04:35, 04:37\*04:20, 04:40, 04:242\*04:238 | \*03:302, 14:45\*03:135 |
| **20** | 165 bp 250 bp 545 bp | \*04:44\*04:47, 04:170N, 04:209\*04:15:02, 04:17, 04:100, 04:178, 04:224, 04:230, 04:242  | \*03:231, 05:78:01-05:78:02, 15:36, **A\*01:118, A\*02:109, A\*33:52** |
| **21** | 85 bp 145 bp 240 bp | \*04:23, 04:108, 04:218\*04:38\*04:39, 04:121 |  |
| **22** | 120 bp 170 bp360 bp | \*04:24, 04:139, 04:140, 04:166, 04:420 \*04:26\*04:226  | \*07:125, 07:356, 07:531\*02:104, 15:100 |
| **23** | 85 bp215 bp235 bp | \*04:25\*04:41\*04:144  | \*03:171, 03:211:01, 05:93, 06:73, 08:20, 08:40, 12:109 |
| **24** | 130 bp170 bp | \*04:24\*04:30, 04:42:01-04:42:02, 04:220  | \*07:125, 07:356, 07:531 |
| **25** | 160 bp200 bp | \*04:163 \*04:43, \*04:94:01-04:94:02, 04:171  | **A\*24:52**\*06:101, 12:10:01-12:10:02, 18:03, **B\*15:27:01-15:27:03, B\*15:109, B\*15:327, B\*15:344, B\*15:398** |
| **26** | 210 bp245 bp | \*04:45, 04:86\*04:250 |  |
| **27** | 125 bp255 bp 280 bp | \*04:50\*04:120\*04:46  | \*05:64:01-05:64:02, 08:19, 08:101, 08:143 |
| **28** | 120 bp160 bp 215 bp255 bp | \*04:75\*04:223:01-04:223:02 \*04:48\*04:204 | \*05:64:01-05:64:02, 08:19 |
| **29** | 105 bp 195 bp245 bp | \*04:82, 04:159\*04:49, 04:132\*04:170N, 04:249 |  |
| **30** | 75 bp125 bp | \*04:53\*04:234N  | \*05:49, **B\*07:90**\*06:152N |
| **31** | 130 bp 170 bp360 bp | \*04:95N, 04:139\*04:51, 04:145\*04:226  | \*02:104, 15:100 |
| **32** | 105 bp 235 bp 275 bp | \*04:78, 04:141\*04:59Q, 04:121\*04:77 | **B\*40:100** |
| **33** | 90 bp 180 bp | \*04:72, 04:218\*04:58, 04:65, 04:160, 04:203N | \*07:08, 07:108:01-07:108:02, **B\*40:100** |
| **34** | 75 bp 200 bp 270 bp | \*04:96\*04:13, 04:58, 04:61, 04:68, 04:160, 04:253N\*04:120, 04:201 | \*07:08, 07:108:01-07:108:02, 08:101, 08:143, **B\*47:09**\*05:64:01-05:64:02, 08:19, 08:101, 08:143 |
| **35** | 85 bp120 bp 145 bp 175 bp | \*04:62\*04:255N\*04:115N\*04:76, 04:137 |  |
| **36** | 115 bp150 bp | \*04:57 \*04:63, 04:117 |  |
| **37** | 110 bp 135 bp | \*04:63, 04:73 \*04:74, 04:125 |  |
| **38** | 95 bp 140 bp | \*04:83, 04:123N\*04:74, 04:117 | \*03:232, 18:07N |
| **39** | 110 bp140 bp 330 bp | \*04:113\*04:71, 04:95N \*04:79 | \*14:71\*01:02:34, 01:21 |
| **40** | 140 bp 205 bp265 bp | \*04:56\*04:86\*04:64:01-04:64:02, 04:201 |  |
| **41** | 135 bp 165 bp280 bp | \*04:105N\*04:131\*04:54  | \*01:04, 01:54, 01:97, 01:102, 14:02:01:01-14:24:01, 14:25, 14:27-14:53, 14:56-14:78, 14:80-14:81 |
| **43** | 170 bp 255 bp | \*04:81, 04:137\*04:88N, 04:209 |  |
| **44** | 90 bp115 bp 140 bp | \*04:108, 04:202 \*04:254\*04:40, 04:60, 04:125, 04:242 | \*03:81, 03:175, 03:199, 03:245, 03:317, 14:24:01, **B\*15:78:03** |
| **45** | 130 bp 165 bp | \*04:66, 04:233N\*04:16, 04:103, 04:26, 04:145  | \*02:104, 03:248, 05:141, 12:194, 15:100, 16:62 |
| **46** | 125 bp160 bp 190 bp 300 bp | \*04:93N\*04:205N, 04:131 \*04:187, 06:135\*04:67 | \*06:128N |
| **47** | 50 bp 295 bp | \*04:114 \*04:146, 04:161  | \*01:59, 01:118, 02:65, 03:130, 03:140, 03:243, 05:20, 06:82, 07:49, 07:210, 07:238, 07:247, 07:403, 12:54, 12:188, 14:04, 14:64, 14:77, 15:85, 16:57, **A\*68:46, B\*07:253****\***15:97, 03:205w |
| **48** | 390 bp415 bp  | \*04:195\*04:84  | \*03:206, 03:212, 08:128 |
| **50** | 295 bp335 bp | \*04:161\*04:162  | \*03:205w |
| **525** | 110 bp185 bp240 bp | \*04:150\*04:203N\*04:155 |  |
| **54** | 140 bp235 bp | \*04:115N, 04:219\*04:249 |  |
| **55** | 190 bp245 bp | \*04:191N, 04:215N, 04:225N\*04:250 |  |
| **56** | 425 bp470 bp | \*04:28\*04:144  | \*03:171, 03:211:01, 05:93, 06:73, 08:20, 08:40, 12:109 |
| **57** | 150 bp245 bp | \*04:205N\*04:182, 04:233N  | \*06:128N\*06:78, 07:309 |
| **58** | 170 bp275 bp | \*04:217N\*04:196 |  |
| **61** | 160 bp285 bp | \*04:236N\*04:206  | \*07:246:02, **A\*11:92** |
| **62** | 105 bp205 bp | \*04:141\*04:253N |  |

**4**The following HLA-C\*04 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |  |  |
| --- | --- | --- | --- |
| Alleles | Primer mix | Alleles | Primer mix |
| C\*04:05, 04:112 | 6 | C\*04:61, 04:96 | 34 |
| C\*04:20, 04:238 | 19 | C\*04:62, 04:76 | 35 |
| C\*04:23, 04:38, 04:39 | 21 | C\*04:65, 04:72 | 33 |
| C\*04:25, 04:41 | 23 | C\*04:67, 04:93N, 04:187 | 46 |
| C\*04:44, 04:47 | 20 | C\*04:79, 04:113 | 39 |
| C\*04:46, 04:50 | 27 | C\*04:81, 04:88N | 43 |
| C\*04:54, 04:105N | 41 | C\*04:114, 04:146 | 47 |
| C\*04:56, 04:64:01-04:64:02 | 40 | C\*04:139, 04:226 | 22 |
| C\*04:59Q, 04:78 | 32 | C\*04:150, 04:155 | 52 |

**5**This lot of the C\*04 subtyping kit cannot distinguish the C\*04:15:02 and the C\*04:224 and 04:230 alleles, or the C\*04:17 and C\*04:100 alleles

‘w’, may be weakly amplified.