Gel Documentation Form and Worksheet

HLA-A\*24 (101.422-24/03, -24u/03u) Lot No: 1E3 Expiry Date: 2019-01-01

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 1, 4, 6 and 48 may give rise to a lower yield of HLA-specific PCR product than the other A\*24 primer mixes, most pronounced in primer mix 1.

Primer mixes 10, 20, 28, and 29 have a tendency to giving rise to primer oligomer formation.

Primer mixes 1, 11, 19, 20, 27, 28, 36, 52, 54, 64, 76, 84 and 93 may have tendencies of unspecific amplifications.

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-A\*24 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 3.23.0, January 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 11: Specific PCR fragment of 200 bp in the A\*24:08, 24:42, 24:89, 24:310:01-24:310:02 and the A\*02:76:01-02:76:02, 02:319 and 30:22 alleles. Specific PCR fragment of 240 bp in the A\*24:61, 24:307 and the A\*23:38N alleles.

Primer mix 14: Specific PCR fragment of 100 bp in the A\*24:09N, 24:267 and the A\*23:18 alleles. Specific PCR fragment of 165 bp in the A\*24:251 allele.

Primer mix 15: Specific PCR fragment of 105 bp in the A\*24:19, 24:44, 24:100, 24:109 and 24:260 alleles. Specific PCR fragment of 385 bp in the A\*24:14-24:15, 24:51, 24:53, 24:57, 24:92, 24:291, 24:296, 24:324 and the A\*02:46, 02:48, 02:70, 02:129, 02:571, 03:30, 03:152, 30:07, 31:08, 32:05, 32:79, 33:21 and 33:53 alleles.

Primer mix 21: Specific PCR fragment of 155 bp in the A\*24:31 allele. Specific PCR fragment of 215 bp in the A\*24:294Q allele.

Primer mix 22: Specific PCR fragment of 125 bp in the A\*24:27 allele. Specific PCR fragment of 180 bp in the A\*24:33 and the A\*02:01:01:01-02:01:14Q, 02:01:17-02:01:19, 02:01:21-02:01:81, 02:01:83-02:06:12, 02:06:14-02:06:15, 02:06:17-02:07:03, 02:07:05-02:22:02, 02:24:01-02:64:02, 02:66-02:68, 02:70-02:97:02, 02:99, 02:101:01-02:131, 02:133-02:134, 02:136-02:178, 02:180-02:304, 02:306-02:454, 02:456-02:468:02N, 02:469w, 02:470-02:610 and 69:01-69:03 alleles.

Primer mix 24: Specific PCR fragment of 215 bp in the A\*24:24, 24:67, 24:145, 24:156 and 24:191 and the A\*23:51, 26:16, 29:37, 29:56, 30:02:01:01-30:04:02, 30:06, 30:09-30:10, 30:12, 30:25, 30:27-30:29, 30:32-30:34, 30:45, 30:47, 30:50, 30:51, 30:57, 30:61, 30:64, 30:66-30:70N, 30:76-30:77, 30:80, 30:84, 30:85, 30:88, 30:90 and 32:07 alleles. Specific PCR fragment of 240 bp in the A\*24:19, 24:28, 24:44 and 24:89 and the A\*02:76:01-02:76:02, 02:319, 30:31 and 30:60 alleles. Specific PCR fragment of 215 and 240 bp in the A\*24:290 and the A\*03:72, 11:88, 30:01:01-30:01:11, 30:11:01-30:11:02, 30:13-30:20, 30:23-30:24, 30:26, 30:30, 30:35-30:44, 30:46, 30:48-30:49, 30:52-30:54, 30:56, 30:58-30:59N, 30:62-30:63, 30:65, 30:71-30:75, 30:78N, 30:81-30:83, 30:86-30:87, 30:89, 30:91-30:97, 68:45 and 68:117 alleles.

Primer mix 25: Specific PCR fragment of 205 bp in the A\*24:13:01-24:13:02, 24:24, 24:56, 24:94, 24:177, 24:188, 24:207, 24:228, 24:315 and the A\*02:17:01-02:17:03, 02:108, 02:110, 02:268, 02:300, 02:303, 23:01:01-23:01:18, 23:03:01-23:65, 23:67-23:69, 29:07, 29:49 and 31:29 and in the B\*18:27 alleles. Specific PCR fragment of 230 bp in the A\*24:204 and 24:213 alleles. Specific PCR fragment of 205 bp and 230 bp in the A\*24:18 allele.

Primer mix 26: Specific PCR fragment of 90 bp in the A\*24:96 and 24:256 alleles. Specific PCR fragment of 220 bp in the A\*24:22, 24:160 and 24:299 alleles.

Primer mix 27: Specific PCR fragment of 80 bp in the A\*24:32 and the A\*23:36, 32:05 and 32:79 alleles. Specific PCR fragment of 370 bp in the A\*24:14 and 24:324 and the A\*02:46, 02:48, 02:70, 02:129 and 02:571 alleles.

Primer mix 28: Specific PCR fragment of 75 bp in the A\*24:155N allele. Specific PCR fragment of 125 bp in the A\*24:266 allele. Specific PCR fragment of 155 bp in the A\*24:35, 24:264 and the A\*11:193 alleles.

Primer mix 29: Specific PCR fragment of 75 bp in the A\*24:155N allele. Specific PCR fragment of 120 bp in the A\*24:265 and 24:271 and the A\*01:45, 03:78 and 11:108 alleles. Specific PCR fragment of 210 bp in the A\*24:08, 24:29, 24:42, 24:89, 24:309-24:310:02 and the A\*02:76:01-02:76:02, 02:319 and 30:22 alleles.

Primer mix 30: Specific PCR fragment of 125 bp in the A\*24:21:01-24:21:03 and 24:208 and the A\*23:03:01-23:03:02, 29:07, 29:49 and 31:29 and in the B\*18:27 alleles. Specific PCR fragment of 200 bp in the A\*24:59, 24:190, 24:210, 24:229 and 24:285 alleles. Specific PCR fragment of 125 bp and 200 bp in the A\*11:139 allele.

Primer mix 31: Specific PCR fragment of 155 bp in the A\*24:37 allele. Specific PCR fragment of 235 bp in the A\*24:04, 24:109, 24:119 and 24:129 and the A\*01:02, 01:20, 01:188, 01:190, 26:16, 29:37, 29:56, 30:57 and 30:90 alleles.

Primer mix 35: Specific PCR fragment of 180 bp in the A\*24:142 allele. Specific PCR fragment of 205 bp in the A\*24:45N and 24:88 alleles.

Primer mix 36: Specific PCR fragment of 115 bp in the A\*24:47 and 24:124 and the A\*31:07 and 31:10 alleles. Specific PCR fragment of 220 bp in the A\*24:192 and in the B\*49:27 alleles. Specific PCR fragment of 245 bp in the A\*24:232N allele.

Primer mix 38: Specific PCR fragment of 145 bp in the A\*24:49 allele. Specific PCR fragment of 170 bp in the A\*24:137 allele. Specific PCR fragment of 230 bp in the A\*24:126 allele.

Primer mix 39: Specific PCR fragment of 75 bp in the A\*24:185N allele. Specific PCR fragment of 210 bp in the A\*24:50 and the A\*02:129 alleles.

Primer mix 40: Specific PCR fragment of 95 bp in the A\*24:02:03Q and the A\*01:01:38L alleles. Specific PCR fragment of 225 bp in the A\*24:08, 24:20 and 24:88 alleles.

Primer mix 41: Specific PCR fragment of 200 bp in the A\*24:26, 24:62 and 24:314 and the A\*01:39, 01:145, 23:45, 26:29, 26:49, 31:05, 32:13, 33:10 and 66:10 alleles. Specific PCR fragment of 240 bp in the A\*24:118 and 24:132N alleles.

Primer mix 42: Specific PCR fragment of 95 bp in the A\*24:312N allele. Specific PCR fragment of 160 bp in the A\*24:22, 24:160, 24:276 and 24:299 and the A\*02:262 and 02:547 alleles. Specific PCR fragment of 190 bp in the A\*24:24, 24:67, 24:169 and 24:290 and the A\*26:16, 68:45 and 68:117 and in the C\*04:81 alleles.

Primer mix 45: Specific PCR fragment of 150 bp in the A\*24:276 allele. Specific PCR fragment of 200 bp in the A\*24:113 allele. Specific PCR fragment of 240 bp in the A\*24:14, 24:52, 24:132N, 24:233, 24:291 and 24:324 and the A\*01:20, 01:66, 01:73, 01:130, 02:19, 02:36-02:37, 02:54, 02:255, 02:417, 26:29, 26:49 and 66:10 and weakly in the B\*07:64, B\*15:12, 15:19, 15:270, 15:298 and 15:304N alleles.

Primer mix 47: Specific PCR fragment of 195 bp in the A\*24:18, 24:56, 24:123 and 24:177 alleles. Specific PCR fragment of 410 bp in the A\*24:26 and the A\*03:30, 03:152, 30:07, 31:08, 32:05, 32:79 and 33:53 alleles.

Primer mix 49: Specific PCR fragment of 165 bp in the A\*24:159 allele. Specific PCR fragment of 250 bp in the A\*24:23 and 24:220 and in the B\*18:27 alleles.

Primer mix 50: Specific PCR fragment of 165 bp in the A\*24:251 allele. Specific PCR fragment of 225 bp in the A\*24:34, 24:252N and 24:270 alleles. Specific PCR fragment of 260 bp in the A\*24:163N and 24:255 and the A\*23:27 alleles.

Primer mix 51: Specific PCR fragment of 165 bp in the A\*24:140 allele. Specific PCR fragment of 215 bp in the A\*24:38 allele.

Primer mix 52: Specific PCR fragment of 75 bp in the A\*24:293 allele. Specific PCR fragment of 210 bp in the A\*24:264 and the A\*11:193 alleles. Specific PCR fragment of 240 bp in the A\*24:40N allele.

Primer mix 53: Specific PCR fragment of 125 bp in the A\*24:158N allele. Specific PCR fragment of 160 bp in the A\*24:303N allele. Specific PCR fragment of 230 bp in the A\*24:55 and 24:315 alleles.

Primer mix 54: Specific PCR fragment of 175 bp in the A\*24:58 and 24:169 and in the C\*04:81 alleles. Specific PCR fragment of 285 bp in the A\*24:248 and in the C\*03:231, C\*04:52, 04:55, C\*14:10 and 14:46:02 alleles.

Primer mix 55: Specific PCR fragment of 80 bp in the A\*24:205 allele. Specific PCR fragment of 240 bp in the A\*24:60N and 24:307 alleles. Specific PCR fragment of 275 bp in the A\*24:173 allele.

Primer mix 59: Specific PCR fragment of 240 bp in the A\*24:68 and 24:143 alleles. Specific PCR fragment of 280 bp in the A\*24:127, 24:215 and 24:288 alleles.

Primer mix 60: Specific PCR fragment of 140 bp in the A\*24:250 allele. Specific PCR fragment of 210 bp in the A\*24:183N and 24:270 alleles. Specific PCR fragment of 275 bp in the A\*24:69 allele.

Primer mix 61: Specific PCR fragment of 105 bp in the A\*24:70 allele. Specific PCR fragment of 165 bp in the A\*24:323N allele. Specific PCR fragment of 280 bp in the A\*24:127, 24:141 and 24:288 alleles.

Primer mix 62: Specific PCR fragment of 120 bp in the A\*24:72 and 24:91 and the A\*02:106 and 02:145 alleles. Specific PCR fragment of 180 bp in the A\*24:275 allele. Specific PCR fragment of 280 bp in the A\*24:297 and the A\*23:16 and 68:107 alleles.

Primer mix 65: Specific PCR fragment of 80 bp in the A\*24:75 allele. Specific PCR fragment of 155 bp in the A\*24:194 allele. Specific PCR fragment of 180 bp in the A\*24:275 allele. Specific PCR fragment of 235 bp in the A\*24:90:01N-24:90:02N and the A\*02:88N alleles.

Primer mix 67: Specific PCR fragment of 90 bp in the A\*24:249 and 24:267 and the A\*23:18 alleles. Specific PCR fragment of 230 bp in the A\*24:79 allele.

Primer mix 68: Specific PCR fragment of 230 bp in the A\*24:143 allele. Specific PCR fragment of 280 bp in the A\*24:78, 24:141 and 24:196 alleles.

Primer mix 69: Specific PCR fragment of 115 bp in the A\*24:80 and 24:225:01-24:225:02 and in the C\*14:14 alleles. Specific PCR fragment of 265 bp in the A\*24:184 and 24:240N alleles.

Primer mix 70: Specific PCR fragment of 110 bp in the A\*24:134 allele. Specific PCR fragment of 200 bp in the A\*24:96 and 24:146 alleles. Specific PCR fragment of 250 bp in the A\*24:81 allele.

Primer mix 71: Specific PCR fragment of 125 bp in the A\*24:128 and 24:269 and the A\*23:29 alleles. Specific PCR fragment of 205 bp in the A\*24:183N and 24:199 alleles. Specific PCR fragment of 260 bp in the A\*24:82 and the A\*29:13, 31:07-31:08, 31:10 and 32:42 alleles.

Primer mix 73: Specific PCR fragment of 100 bp in the A\*24:84N allele. Specific PCR fragment of 170 bp in the A\*24:131, 24:138, 24:188 and 24:218 and the A\*02:17:01-02:17:03, 02:108, 02:110, 02:268, 02:300 and 02:303 alleles.

Primer mix 74: Specific PCR fragment of 155 bp in the A\*24:180 and 24:323N alleles. Specific PCR fragment of 195 bp in the A\*24:85 and 24:197 alleles.

Primer mix 75: Specific PCR fragment of 145 bp in the A\*24:180 allele. Specific PCR fragment of 285 bp in the A\*24:86N, 24:196 and 24:215 alleles.

Primer mix 76: Specific PCR fragment of 175 bp in the A\*24:76 and 24:263 alleles. Specific PCR fragment of 255 bp in the A\*24:98 and 24:220 and in the B\*18:27 alleles.

Primer mix 77: Specific PCR fragment of 145 bp in the A\*24:122 allele. Specific PCR fragment of 210 bp in the A\*24:06, 24:87, 24:138, 24:167, 24:285, 24:289 and the A\*23:02 and 23:66 alleles.

Primer mix 79: Specific PCR fragment of 195 bp in the A\*24:182 allele. Specific PCR fragment of 250 bp in the A\*24:14, 24:93, 24:114, 24:233 and 24:324 and the A\*01:20, 01:66, 01:130, 02:19, 02:36-02:37, 02:54, 02:255 and 02:417 alleles.

Primer mix 80: Specific PCR fragment of 175 bp in the A\*24:140 and 24:142 alleles. Specific PCR fragment of 220 bp in the A\*24:95 allele.

Primer mix 81: Specific PCR fragment of 100 bp in the A\*24:139 allele. Specific PCR fragment of 210 bp in the A\*24:97 and 24:133 alleles.

Primer mix 82: Specific PCR fragment of 185 bp in the A\*24:99 and 24:130 alleles. Specific PCR fragment of 225 bp in the A\*24:125 allele. Specific PCR fragment of 285 bp in the A\*24:248 and in the C\*03:231, C\*04:52, 04:55, C\*14:10 and 14:46:02 alleles.

Primer mix 83: Specific PCR fragment of 125 bp in the A\*24:111 and 24:225:01-24:225:02 and in the C\*14:14 alleles. Specific PCR fragment of 270 bp in the A\*24:101, 24:173 and 24:240N alleles.

Primer mix 84: Specific PCR fragment of 95 bp in the A\*24:103 allele. Specific PCR fragment of 210 bp in the A\*24:107 allele. Specific PCR fragment of 290 bp in the A\*24:102 and 24:287 alleles.

Primer mix 85: Specific PCR fragment of 145 bp in the A\*24:117 allele. Specific PCR fragment of 210 bp in the A\*24:104 and the A\*03:30, 03:152, 23:64, 32:05 and 32:79 alleles.

Primer mix 86: Specific PCR fragment of 190 bp in the A\*24:121 and 24:182 alleles. Specific PCR fragment of 250 bp in the A\*24:105 and 24:222N allele.

Primer mix 87: Specific PCR fragment of 90 bp in the A\*24:17, 24:41, 24:62, 24:106, 24:208 and 24:330 and the A\*11:139, 23:31, 23:45, 23:53, 29:07, 29:49 and 31:29 alleles. Specific PCR fragment of 120 bp in the A\*24:110 allele. Specific PCR fragment of 190 bp in the A\*24:136 and 24:197 alleles.

Primer mix 88: Specific PCR fragment of 75 bp in the A\*24:185N allele. Specific PCR fragment of 170 bp in the A\*24:116 and 24:137 alleles.

Primer mix 89: Specific PCR fragment of 75 bp in the A\*24:07:01-24:07:02, 24:108, 24:131 and 24:288 and the A\*23:13 alleles. Specific PCR fragment of 220 bp in the A\*24:192 and in the B\*49:27 alleles. Specific PCR fragment of 270 bp in the B\*51:129 alleles. Specific PCR fragment of 75 bp and 270 bp in the A\*24:112 allele.

Primer mix 90: Specific PCR fragment of 100 bp in the A\*24:115 and 24:206 alleles. Specific PCR fragment of 180 bp in the A\*24:168 allele. Specific PCR fragment of 290 bp in the A\*24:234 and 24:287 and the A\*23:47 alleles.

Primer mix 91: Specific PCR fragment of 70 bp in the A\*24:135:01-24:135:02 and the A\*23:45 alleles. Specific PCR fragment of 270 bp in the A\*24:238 allele.

Primer mix 93: Specific PCR fragment of 170 bp in the A\*24:74:01-24:74:02 and in the B\*44:63w alleles. Specific PCR fragment of 270 bp in the A\*24:112 and in the B\*51:129 alleles.

Primer mix 94: Specific PCR fragment of 105 bp in the A\*24:206 allele. Specific PCR fragment of 220 bp in the A\*24:278N allele.

Primer mix 95: Specific PCR fragment of 115 bp in the A\*24:269 allele. Specific PCR fragment of 145 bp in the A\*24:231 and 24:250 alleles. Specific PCR fragment of 260 bp in the A\*24:163N allele.

**4**The A\*24 primer set cannot separate the A\*24:164 and A\*33:19 alleles. These two alleles can be distinguished by the HLA-A low resolution and/or HLA-A\*33 kits.

**5**The following HLA-A\*24 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |  |  |
| --- | --- | --- | --- |
| **Alleles** | **Primer mix** | **Alleles** | **Primer mix** |
| A\*24:29, 24:271 | 29 | A\*24:84N, 24:218 | 73 |
| A\*24:37, 24:119 | 31 | A\*24:101, 24:111 | 83 |
| A\*24:47, 24:232N | 36 | A\*24:102, 24:103 | 84 |
| A\*24:49, 24:126 | 38 | A\*24:104, 24:117 | 85 |
| A\*24:60N, 24:205 | 55 | A\*24:105, 24:121 | 86 |
| A\*24:72, 24:297 | 62 | A\*24:106, 24:136 | 87 |
| A\*24:79, 24:249 | 67 | A\*24:115, 24:234 | 90 |
| A\*24:81, 24:134 | 70 | A\*24:135:01-24:135:02, 24:238 | 91 |
| A\*24:82, 24:128 | 71 | A\*24:225:01-24:225:02, 24:240N | 69, 83 |

The HLA-A\*24 subtyping kit cannot distinguish the silent mutations in the A\*24:02:01:01, 24:02:01:03-24:02:04, 24:02:06-24:02:09, 24:02:11-24:02:12, 24:02:14-24:02:64, 24:02:66-24:02:88, 24:02:90-24:02:96 and 24:226:01-24:226:02 alleles, the A\*24:02:05 and 24:02:89 alleles, the A\*24:02:10 and 24:02:65 alleles, the A\*24:03:01-24:03:03 alleles, the A\*24:07:01-24:07:02 alleles, the 24:10:01-24:10:02 alleles, the A\*24:21:01-24:21:03 alleles, the A\*24:74:01-24:74:02, the A\*24:90:01N-24:90:02N alleles, the A\*24:135:01-24:135:02, the A\*24:225:01-24:225:02 alleles or the A\*24:310:01-24:310:02 alleles.

‘w’, might be weakly amplified.

Change in revision R01 compared to R00:

1. The B\*07:64, B\*15:12, 15:19, 15:270, 15:298 and 15:304N alleles are all amplified by primer mix 32 and weakly amplified by primer mix 45. This has been corrected in the Interpretation and Specificity Tables.

Change in revision R02 compared to R01:

1. Primer mix 29 does not amplify the A\*02:453, 02:557 and 66:17 alleles. This has been corrected in the Specificity and Interpretation Tables.