Gel Documentation Form and Worksheet

HLA-A\*68 (101.418-12/04 -12u/04u) Lot No: 4E1 Expiry Date: 2019-05-01

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, 17, 29 and 31 may give rise to a lower yield of HLA-specific PCR product than the other HLA-A\*68 primer mixes.

Primer mixes 20, 35, 38 and 47may have tendencies of unspecific amplifications.

Primer mix 32 has a tendency to giving rise to primer oligomer formation.

Primer mix 11 may faintly amplify the A\*02:06:05, 02:06.09, 02:122, 11:70, 11:121 and 26:07:01-26:07:02 alleles.

Primer mix 48 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\*68 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>.

Primer mix 4: Specific PCR fragment of 180 bp in the A\*68:30 allele. Specific PCR fragment of 225 bp in the A\*68:03:01-68:03:02, 68:05 and 68:20 alleles. Specific PCR fragment of 180 bp and 225 bp in the A\*02:06:05 allele.

Primer mix 5: Specific PCR fragment of 140 bp in the A\*68:25 and the A\*02:339, 02:407, 02:449 and 02:594 alleles. Specific PCR fragment of 235 bp in the A\*68:04:01-68:04:02 and the A\*11:98, 33:51 and 66:15 alleles.

Primer mix 8: Specific PCR fragment of 160 bp in the A\*68:07 allele. Specific PCR fragment of 240 bp in the A\*68:46 allele.

Primer mix 9: Specific PCR fragment of 195 bp in the A\*68:47 allele. Specific PCR fragment of 250 bp in the A\*68:08:01-68:08:02 and 68:63 and the A\*25:19:01-25:19:02, 25:30, 26:43:01-26:43:02, 26:112, 34:03?-34:04?, 34:06?-34:10N?, 34:13? and 66:06 alleles.

Primer mix 10: Specific PCR fragment of 165 bp in the A\*68:09, 68:26, 68:129 and 68:134 and the A\*01:12, 01:19, 01:127, 01:136, 03:10, 03:167, 11:01:01:01-11:01:34, 11:01:36-11:01:56, 11:01:58-11:07, 11:10-11:21N, 11:26-11:27, 11:29-11:30, 11:32:01-11:34, 11:36-11:43, 11:45-11:49, 11:51-11:52Q, 11:54-11:58, 11:60-11:93, 11:95-11:111, 11:114-11:115N, 11:117-11:129, 11:131-11:139, 11:141-11:157, 11:159-11:161, 11:163-11:175, 11:177-11:182Q, 11:184-11:190, 11:192-11:203, 11:205-11:210N, 11:212-11:215N, 11:217-11:225, 11:227-11:228, 11:230-11:245, 24:17, 24:41, 24:208, 24:296, 29:05, 29:33, 29:77 and 29:87 and in the C\*16:67 alleles. Specific PCR fragment of 190 bp in the A\*68:148Q allele.

Primer mix 11: Specific PCR fragment of 160 bp in the A\*68:17 allele. Specific PCR fragment of 200 bp in the A\*68:10, A\*68:14 and 68:120N and the A\*02:358 alleles.

Primer mix 12: Specific PCR fragment of 200 bp in the A\*68:38, 68:110 and 68:120N and in the C\*06:139 alleles. Specific PCR fragment of 405 bp in the A\*68:11N allele.

Primer mix 13: Specific PCR fragment of 210 bp in the A\*68:12, 68:50, 68:117, 68:129 and 68:131 and the A\*26:122 alleles. Specific PCR fragment of 240 bp in the A\*68:51 and the A\*26:18 alleles. Specific PCR fragment of 260 bp in the A\*68:113 and 68:115 alleles.

Primer mix 15: Specific PCR fragment of 145 bp in the A\*68:19 and the A\*02:237, 03:17:01, 03:171, 24:18, 24:204 and 24:213 alleles. Specific PCR fragment of 200 bp in the A\*68:14 and 68:80-68:81 and the A\*02:121, 02:425, 02:517, 23:47, 24:141, 24:234 and 24:339 and in the C\*12:37w alleles.

Primer mix 18: Specific PCR fragment of 200 bp in the A\*68:26, 68:65, 68:115 and 68:131 and the A\*01:01:01:01-01:01:65, 01:01:67-01:04N, 01:06-01:29, 01:31N-01:33, 01:35-01:71, 01:73-01:78, 01:80-01:85, 01:87-01:101, 01:103-01:144, 01:146-01:166, 01:168-01:199, 01:201-01:208Q, 03:18, 03:97, 03:122, 03:135, 03:167, 11:27, 11:38-11:39, 11:94, 11:209, 23:53, 23:70, 24:17, 24:41, 24:208, 24:296, 29:03, 29:33 and 80:01:01:01-80:03 alleles. Specific PCR fragment of 225 bp in the A\*68:21:01-68:21:02 and the A\*03:04:01-03:04:03, 11:153:01-11:153:02 alleles. Specific PCR fragment of 200 bp and 225 bp in the A\*01:86 allele.

Primer mix 21: Specific PCR fragment of 165 bp in the A\*68:28 and the A\*02:49, 24:87, 24:285, 24:289, 24:299, 31:97, 66:19, 66:26Q and in the B\*07:197, B\*27:45 and 27:108 alleles. Specific PCR fragment of 215 bp in the A\*68:24 and 68:123 alleles.

Primer mix 24: Specific PCR fragment of 85 bp in the A\*68:112:01-68:112:02 and the A\*34:15 and in the B\*08:131, 08:136 and 08:139alleles. Specific PCR fragment of 375 bp in the A\*68:105 and the A\*02:55, 26:22, 33:22, 66:09 and 69:01-69:03 alleles.

Primer mix 26: Specific PCR fragment of 100 bp in the A\*68:49N allele. Specific PCR fragment of 190 bp in the A\*68:33 and 68:80 and the A\*24:141 and 33:03:17 alleles.

Primer mix 27: Specific PCR fragment of 105 bp in the A\*68:18N allele. Specific PCR fragment of 185 bp in the A\*68:34 and the A\*02:385 and 02:529 alleles. Specific PCR fragment of 235 bp in the A\*68:43:01-68:43:02 and 11:219 alleles.

Primer mix 28: Specific PCR fragment of 200 bp in the A\*68:35, 68:38 and 68:110 and in the C\*06:139 alleles. Specific PCR fragment of 230 bp in the A\*68:44 allele.

Primer mix 29: Specific PCR fragment of 80 bp in the A\*68:42, 68:54, 68:61 and 68:63 and in the A\*02:03:01-02:03:08, 02:26, 02:99, 02:117, 02:148, 02:171:02, 02:253, 02:258, 02:264, 02:281, 02:315, 02:323, 02:345, 02:355, 02:370, 02:393, 02:402, 02:412, 02:427, 02:431, 02:447, 02:463, 02:466, 02:480, 02:489, 02:505, 02:529, 02:541, 02:544, 02:557, 02:568, 02:589, 02:592, 02:595, 02:612, 02:633-02:634, 03:01:38, 03:123:02, 11:25:01, 11:191 and 26:01:17 alleles. Specific PCR fragment of 250 bp in the A\*68:36 and 68:37 and in the B\*44:03:19-44:03:20 alleles.

Primer mix 30: Specific PCR fragment of 145 bp in the A\*02:117, 02:135, 25:01:01-25:01:08, 25:02-25:07, 25:10-25:19:01, 25:20-25:27:02, 25:29-25:30, 26:01:01:01, 26:01:02-26:01:14, 26:01:16-26:01:25, 26:01:27-26:01:37, 26:02:01, 26:03:01-26:10, 26:12-26:13, 26:15-26:17, 26:19-26:27, 26:29-26:39, 26:41-26:43:02, 26:45-26:46, 26:48-26:72, 26:74-26:109, 26:111, 26:113, 34:01:01-34:01:02, 34:05, 34:11-34:12, 43:01, 66:01:01-66:01:02, 66:02-66:15, 66:17 and 66:19-66:21 alleles. Specific PCR fragment of 245 bp in the A\*68:37 allele.

Primer mix 34: Specific PCR fragment of 105 bp in the A\*68:18N allele. Specific PCR fragment of 165 bp in the A\*68:55:01-68:55:02 and 68:124 and the A\*03:163 and 03:244 alleles.

Primer mix 35: Specific PCR fragment of 140 bp in the A\*68:59N allele. Specific PCR fragment of 295 bp in the A\*68:93 and 68:97 and the A\*32:73 and in the B\*44:129 alleles.

Primer mix 37: Specific PCR fragment of 145 bp in the A\*68:67 and the A\*34:12 alleles. Specific PCR fragment of 170 bp in the A\*68:123 allele. Specific PCR fragment of 285 bp in the A\*68:26, 68:65, 68:115 and 68:131 and the A\*26:29, 26:49 and 66:10 alleles.

Primer mix 39: Specific PCR fragment of 95 bp in the A\*68:106 allele. Specific PCR fragment of 180 bp in the A\*68:68 allele.

Primer mix 41: Specific PCR fragment of 135 bp in the A\*68:144 and the A\*32:68 alleles. Specific PCR fragment of 200 bp in the A\*68:45 and 68:117 and the A\*24:24, 24:67, 24:290 and 26:16 alleles.

Primer mix 42: Specific PCR fragment of 130 bp in the A\*68:60 and 68:99 and the A\*11:164 alleles. Specific PCR fragment of 245 bp in the A\*68:101 and the A\*03:180 alleles.

Primer mix 45: Specific PCR fragment of 150 bp in the A\*68:41 and the A\*01:126 alleles. Specific PCR fragment of 220 bp in the A\*68:72 alleles. Specific PCR fragment of 255 bp in the A\*68:86 and the A\*26:57 and in the B\*39:104 alleles.

Primer mix 46: Specific PCR fragment of 110 bp in the A\*68:52 and the A\*02:396 alleles. Specific PCR fragment of 495 bp in the A\*68:142N allele.

**4**The following HLA-A\*68 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |
| --- | --- |
| Alleles | Primer mix |
| A:68:52, 68:142N | 46 |
| A\*68:68, 68:106 | 39 |
| A\*68:99, 68:101 | 42 |

The HLA-A\*68 subtyping kit cannot distinguish the following silent mutations: the A\*68:01:01:01-68:01:01:02, 68:01:03-68:01:05, 68:01:07-68:01:12 68:01:14, 68:01:18-68:01:20, 68:01:28 and 68:01:32, the A\*68:01:02:01-68:01:02:03, 68:01:06, 68:01:13, 68:01:15-68:01:16, 68:01:21-68:01:27 and 68:01:30-68:01:31, the 68:01:17 and 68:103:02, the A\*68:02:01:01-68:02:10 and 68:02:12, the A\*68:03:01-68:03:02, the A\*68:27:01-68:27:02, the A\*68:43:01-68:43:02 or the A\*68:112:01-68:112:02 alleles.

 ‘w, may be weakly amplified.

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