Gel Documentation Form and Worksheet

HLA-A\*32 (101.431-12/12u) Lot No: 5E4 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**

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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 2, 4 and 20 may have tendencies of unspecific amplifications.

Primer mixes 8 and 10 may give rise to a lower yield of HLA-specific PCR product than the other A\*32 primer mixes.

Primer mix 30 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\*32 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 3.25.0, 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 2: Specific PCR fragment of 430 bp in the A\*32:01:06, 32:02, 32:10 and 32:22 alleles. Specific PCR fragment of 520 bp in the A\*32:01:07, 32:01:12, 32:04, 32:28, 32:33:02 and 32:66 and the A\*02:81, 02:124, 23:36, 25:19:01-25:19:02 and 25:30 alleles.

Specific PCR fragment of 430 bp and 520 bp in the A\*32:01:01-32:01:05, 32:01:08-32:01:11, 32:01:13-32:01:23, 32:05-32:09, 32:11Q-32:21, 32:23-32:27N, 32:29-32:33:01, 32:34-32:65, 32:67-32:77, 32:79-32:83 and 32:85-32:86 and the A\*29:13 allele.

Primer mix 6: Specific PCR fragment of 120 bp in the A\*32:20 allele. Specific PCR fragment of 520 bp in the A\*32:04 and 32:52 and the A\*03:152, 03:219, 24:18, 24:204 and 24:213 alleles.

Primer mix 8: Specific PCR fragment of 165 bp in the A\*32:19N allele. Specific PCR fragment of 215 bp in the A\*32:07 and the A\*01:02w, 01:20w, 03:72, 11:88, 23:09w, 23:51, 24:24, 24:67, 24:145, 24:156, 24:191, 24:290, 26:16, 29:37, 29:56, 30:01:01-30:04:02, 30:06, 30:09-30:20, 30:23-30:30, 30:32-30:54, 30:56-30:59N, 30:61-30:78N, 30:80-30:99, 30:102, 68:45 and 68:117 alleles.

Primer mix 11: Specific PCR fragment of 120 bp in the A\*32:20 allele. Specific PCR fragment of 165 bp in the A\*32:09 allele.

Primer mix 12: Specific PCR fragment of 130 bp in the A\*32:10 and the A\*02:507, 29:28, 29:79, 31:30, 31:97 and 33:94 and in the B\*07:02:40, C\*02:02:15 and C\*04:175 alleles. Specific PCR fragment of 195 bp in the A\*32:16 allele.

Primer mix 13: Specific PCR fragment of 155 bp in the A\*32:11Q allele. Specific PCR fragment of 200 bp in the A\*32:15 and the A\*01:51, 02:55, 03:24, 25:03, 25:30, 26:20, 34:08 and 68:71 alleles.

Primer mix 15: Specific PCR fragment of 165 bp in the A\*32:13 and the A\*23:03:01, 24:21:03, 24:208, 29:03, 29:33, 31:05 and 33:10 alleles. Specific PCR fragment of 220 bp in the A\*32:18 allele.

Primer mix 17: Specific PCR fragment of 75 bp in the A\*32:21 and the A\*29:62, 31:45 and 33:16 alleles. Specific PCR fragment of 200 bp in the A\*32:44 and the A\*33:75 alleles.

Primer mix 18: Specific PCR fragment of 130 bp in the A\*32:24 allele. Specific PCR fragment of 185 bp in the A\*32:60 allele. Primer mix 19: Specific PCR fragment of 110 bp in A\*32:25 and 32:45N alleles. Specific PCR fragment of 185 bp in A\*32:60 allele.

Primer mix 20: Specific PCR fragment of 125 bp in the A\*32:23 and the A\*33:46 alleles. Specific PCR fragment of 220 bp in the A\*32:54 and the A\*02:294, 34:01:01?-34:01:02?, 34:05? and 66:08 alleles.

Primer mix 22: Specific PCR fragment of 175 bp in the A\*32:56N allele. Specific PCR fragment of 230 bp in the A\*32:27N allele.

Primer mix 23: Specific PCR fragment of 80 bp in A\*32:28 and 32:66 and the A\*02:41, 02:80, 02:117, 02:289:01, 02:304, 02:454, 23:45, 24:62, 26:10, 31:67-31:68 and 33:32:01 alleles. Specific PCR fragment of 225 bp in the A\*32:53 and the A\*02:480 and 33:39 alleles.

**4**The following HLA-A\*32 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

|  |  |
| --- | --- |
| Alleles | Primer mix |
| A\*32:10, 32:16 | 12 |
| A\*32:21, 32:44 | 17 |
| A\*32:23, 32:54 | 20 |
| A\*32:28, 32:53 | 23 |

The HLA-A\*32 subtyping kit cannot distinguish the following silent mutations: the A\*32:01:01-32:01:17, 32:01:19-32:01:23 and the 32:55:01-32:55:02 alleles.

‘w’, might be weakly amplified.

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

Change in revision R01 compared to R00:

1. Primer mix 14 does not amplify the A\*32:12 allele. This has been corrected in the Interpretation and Specificity Tables.