Gel Documentation Form and Worksheet

HLA-A low resolution screening Lot No: 1F4 Expiry Date: 2019-10-01

(101.403-48/12,-48u/12u)

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.

The primer pairs in wells 1, 2, 5, 7, 8, 10, 11, 14, 15 and 18 will in many samples give rise to two or three HLA-specific PCR fragments.

Primer mix 3 may faintly amplify the A\*30:04:01-30:04:02, 30:06, 30:17, 30:29, 30:46, 30:77, 30:90, 30:99 and 30:103 alleles.

Primer mix 9 may weakly amplify the A\*34 alleles.

Primer mixes 10, 14 and 20 have a tendency to giving rise to primer oligomer formation.

Primer mix 22 might faintly amplify most A\*11 alleles.

Primer mix 15 may give rise to a lower yield of HLA-specific PCR product than the other HLA-A low primer mixes.

Primer mix 3 and 15 may have tendency of unspecific amplification.

Primer mix 21 may generate a false positive band of about 560 base pairs. This band should be disregarded when interpreting HLA-A low resolution typings.

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.











**1**HLA-A alleles listed on the IMGT/HLA web page 2016-October-17, release 3.26.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

**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 serological reactivity of all HLA-A alleles is not known. In this table we use the expert-assigned serological grouping in Tissue Antigens (2009) **73**:95-170 and the serological grouping of the sequence-defined allele.

**4**The A\*23:14:01-23:14:02 and the A\*24:24, 24:71 and 24:315 alleles will give rise to identical amplification patterns. These alleles can be separated by the respective high resolution SSP primer sets.

The A\*23:57 and 23:66 and the A\*24:94, 24:138, 24:188 and 24:228 alleles will give rise to identical amplifications patterns. These alleles can be separated by the respective high resolution SSP primer sets.

The 30:01:01-30:02:11, 30:02:13-30:04:02, 30:06-30:07, 30:09-30:20, 30:22-30:54, 30:56-30:88, 30:90-30:100, 30:102-30:104 and 30:106-30:107 and the B\*07:260 alleles will give rise to identical amplifications patterns. These alleles can be separated by the respective high resolution SSP primer sets.

The A\*66:01:01:01-66:01:01:02, 66:01:03, 66:04-66:05, 66:07-66:08, 66:11, 66:13-66:14, 66:17-66:20, 66:22, 66:24 and 66:27N and the A\*26:92 alleles will give rise to identical amplifications patterns. These alleles can be separated by the respective high resolution SSP primer sets.

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

|  |  |
| --- | --- |
| Alleles | Primer mix |
| A\*01:26, 01:136, A\*11:94, 11:112, 11:211 | 11 |
| A\*24:14:01:01-24:15, 24:51-24:53, 24:57, 24:64, 24:114, 24:222N, 24:291, 24:296, 24:304, 24:316, 24:324, C\*04:01:03 | 5 |
| A\*68:01:32, 68:11N | 22 |

‘w’, might be weakly amplified.

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

Change in revision R01 compared to R00:

1. The section “Uniquely Identified Alleles”, the Interpretation Table and the footnotes to the Interpretation Table have been corrected with respect to alleles not possible to separate.

Change in revision R02 compared to R01:

1. Primer mix 21 may generate a false positive band of about 560 base pairs. This band should be disregarded when interpreting HLA-A low resolution typings. A footnote has been added in the Specificity Table.

Change in revision R03 compared to R02:

1. The reactivities of primer mix 17 have been corrected in the Specificity and Interpretation Tables.

Change in revision R04 compared to R03:

1. Primer mix 4 amplifies the A\*01:15N allele. This has been corrected in the Specificity and Interpretation Tables.