Gel Documentation Form and Worksheet

HLA-A low resolution Lot No: 3E4 Expiry Date: 2019-03-01

(101.401-48/12,-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.

Primer mix 3 may faintly amplify the A\*30:04:01-30:04:02, 30:06, 30:17, 30:29, 30:46, 30:77, 30:89 and 30:90 alleles.

Primer mix 6 may give rise to a lower yield of A\*23 alleles than the other A low primer mixes.

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

Primer mix 30 may generate a false positive band of about 500 base pairs. This band should be disregarded when interpreting HLA-A low resolution typings.

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

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

Primer mixes 15, 27 and 28 may give rise to a lower yield of HLA-specific PCR product than the other HLA-A low primer mixes.

Primer mixes 6, 10, 14, 20, 21, 23 and 30 have a tendency to giving rise to primer oligomer formation.

Primer mixes 15 and 29 may have tendencies of unspecific amplifications.

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 32 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-July-14, release 3.25.0, [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\*24:71 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 A\*31:08 and A\*33:53 alleles will give rise to identical amplifications. 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\*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, C\*04:01:03 | 5 |

‘ser’, serological HLA specificity.

‘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 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 R02 compared to R01:

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