Gel Documentation Form and Worksheet

HLA-A-B-DR SSP Combi Tray Lot No: 0E4 Expiry Date: 2018-12-01

(101.701-24/06 – 24u/06u)

Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sample ID:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

DNA Conc.(ng/ul):\_\_\_\_\_\_\_\_\_\_

Test Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Tested By:

Review Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Reviewed By:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

***Interpretation:\_\_\_\_\_\_\_\_\_\_\_ Failed lanes: \_\_\_\_\_\_\_\_\_\_\_ Comments:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_***

**Gel Picture**

|  |
| --- |
| PHOTO DOCUMENT |

**HLA-A low**  **resolution**



**HLA-B low**  **resolution**





**DR low**  **resolution**



‘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-A low resolution primer set**

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 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, 30:09, 30:17, 30:29, 30:46, 30:77 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 22 might faintly amplify most A\*11 alleles.

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

Primer mixes 6, 15 and 24 may give rise to a lower yield of HLA-specific PCR product than the other HLA-A low primer mixes.

Primer mixes 15, 20 and 24 may have tendencies of unspecific amplifications.

In primer mix 6 the positive control band may be weaker than for other HLA-A low primer mixes.

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.

**HLA-B low resolution primer set**

HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

Primer mix 28 may give rise to a lower yield of HLA-specific PCR product than the other HLA-B low resolution primer mixes in B\*40, B\*41, B\*45, B\*49 and B\*50 alleles.

Primer mixes 28, 29, 33, 42, 53 and 63 may give rise to a lower yield of HLA-specific PCR product than the other HLA-B low resolution primer mixes.

Primer mix 32 and 33 has pronounced tendencies of unspecific amplifications.

Primer mixes 25, 43, 44, 50, and 69 have a tendency to giving rise to primer oligomer formation.

The B\*57 and B\*58 alleles might be faintly amplified by primer mix 33.

The C\*17:01 to C\*17:04 alleles might be faintly amplified by primer mix 49.

Primer mixes 56 and 67 may generate a false positive band of about 800 base pairs. This band should be disregarded when interpreting HLA-B low resolution typings.

Primer mix 63 may give rise to a lower yield of B\*54 alleles than the other B low primer mixes.

The Bw4-associated HLA-A specificities A9, A23, A24, A2403, A25 and A32 are not amplified by the primer pair in primer mix 70.

**DR low resolution primer set**

HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

Individual alleles can give rise to two differently sized specific PCR fragments in primer mixes 73, 75, 77 to 83, 85, 86, 88, 89, and 91 to 94.

Primer mix 73, 80, 87, 88 and 90 to 94 may have a tendency to giving rise to primer oligomer formation.

Primer mix 91 has a tendency of primer oligomer formation and also has an intense primer cloud due to the high number of primers present in the primer mix.

Primer mixes 75, 76, 80, 81 and 92 may have tendencies of unspecific amplifications.

Due to sharing of sequence motifs in codon 38 and 47, DRB3\*0114 will also be amplified in primer mixes 77, 78 and 89, and DRB3\*01:23 and \*02:32 in mix 77, in addition to primer mix 93.

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.

**HLA-A low resolution Interpretation Table**









**1**HLA-A, HLA-B and HLA-DRB alleles listed on the IMGT/HLA web page 2016-January-19, release 3.23.0, [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. The grouping of not serologically defined alleles is taken from Tissue Antigens 73, 95-170, 2009.

**4**The A\*01:136 and 01:192 and the A\*11:94, 11:112 and 11:211 alleles may be distinguished by the different sizes of the specific PCR products generated by primer mixes 1 and 11.

The A\*24:14-24:15, 24:51-24:53, 24:57, 24:64, 24:114, 24:222N, 24:291, 24:296, 24:304, 24:316 and 24:324 and the C\*04:01:03 alleles may be distinguished by the different sizes of the specific PCR products generated by primer mix 5.

The A\*68:01:32 and 68:11N alleles may be distinguished by the different sizes of the specific PCR products generated by primer mix 22.

The A\*68:08:02 and 68:105 alleles may be distinguished by the different sizes of the specific PCR products generated by primer mix 21.

**5**The HLA-A alleles will be grouped into their corresponding serological specificities, except that following alleles give rise to identical amplification patterns.

These alleles can be separated by the respective high resolution SSP primer sets

|  |  |
| --- | --- |
| **Alleles** | **Alleles** |
| A\*23:14:01-23:14:02, A\*24:24, 24:71, 24:315 | A\*30:01:01-30:04:02, 30:06-30:07, 30:09-30:20, 30:22-30:54, 30:56-30:88, 30:90-30:97, B\*07:260 |
| A\*23:57, 23:66, A\*24:94, 24:138, 24:188, 24:228 | A\*31:08, A\*33:53 |

‘w’, might be weakly amplified.

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

**HLA-B low resolution Interpretation Table**





 



















 





















 











  

**1**HLA-A, HLA-B and HLA-DRB alleles listed on the IMGT/HLA web page 2016-January-19, release 3.23.0, [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-B alleles is not known. The grouping of not serologically defined alleles is taken from Tissue Antigens 73, 95-170, 2009.

page <http://hla.alleles.org/alleles/deleted.html>.

**4**The HLA-B alleles will be grouped into their corresponding serological specificities, except that following alleles give rise to identical amplification patterns.

These alleles can be separated by the respective high resolution SSP primer sets.

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| --- | --- |
| **Alleles** | **Alleles** |
| B\*08:26:01-08:26:02, 08:50, 08:62, 08:85, 08:94, 08:146, B\*42:07 | B\*53:30, B\*57:45, 57:51, 57:69 |
| B\*13:21, 13:35, 13:59, 13:71, B\*44:135, 44:158, 44:184 | B\*55:04, 55:08, 55:13, 55:27, 55:46, 55:49, B\*56:01:05, 56:15, 56:19N, 56:22 |
| B\*14:08:01-14:08:02, B\*39:01:19, 39:25N, 39:30, 39:32-39:34, 39:47, 39:50, 39:74, 39:82, 39:102, 39:107, 39:112 | B\*55:23, 55:32, B\*56:18, 56:31-56:32, 56:50 |
| B\*18:29, 18:72, 18:92, 18:102, B\*35:32:01-35:32:02, 35:37, 35:53N, 35:64:01-35:64:02, 35:68:01-35:68:02, 35:99, 35:118-35:119, 35:174 | B\*57:67:01, B\*58:36 |

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

‘w’, might be weakly amplified.

**DR low resolution Interpretation Table**







**1**HLA-A, HLA-B, HLA-DRB alleles listed on the IMGT/HLA web page 2016-January-19, release 3.23.0. [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 DRB alleles is not known. The grouping of not serologically defined alleles is taken from Tissue Antigens 73, 95-170, 2009.

**4**The HLA-DRB1, -DRB3, -DRB4 and –DRB5 alleles will be grouped into their corresponding serological specificities, except that following alleles give rise to identical amplification patterns:

|  |
| --- |
| **Alleles** |
| DRB1\*08:20, DRB1\*13:18, 13:47, 13:55, 13:158, 13:164 |
| DRB1\*11:149, DRB1\*13:05:01-13:05:03, 13:07:01-13:07:02, 13:11:01-13:11:02, 13:14:01-13:14:03, 13:21:01-13:21:02, 13:42, 13:46, 13:49-13:50:03, 13:62, 13:100, 13:108, 13:132, 13:136, 13:150, 13:169, 13:189, 13:199, 13:203 |
| DRB1\*13:13, 13:119, 13:154, 13:156, DRB1\*14:84, 14:116, 14:144 |

‘w’, might be weakly amplified.

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

Changes in revision R01 compared to R00:

1. Due to sharing of sequence motifs in codon 38 and 47, DRB3\*01:14 will also be amplified in primer mixes 77, 78 and 89 , and DRB3\*01:23 and \*02:32 in mix 77, in addition to primer mix 93.
2. The DRB1\*13:02:02 allele is amplified in primer mix 78.

The Specificity and Interpretation Tables have been changed.

Changes in revision R02 compared to R01:

1. Primer mix 75 does not amplify the DRB1\*16:05:01-16:05:02 and 16:07 alleles. This has been corrected in the Specificity and Interpretation Tables.

Changes in revision R03 compared to R02:

1. Primer mix 34 amplifies the C\*02:06 and C\*15:42 alleles and primer mix 65 amplifies the B\*35:291 and the C\*15:15 alleles. This has been corrected in the Specificity and Interpretation Tables.
2. 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.

Changes in revision R04 compared to R03:

1. Primer mix 32 may have a most pronounced tendency of unspecific amplifications. A footnote has been added in the specificity table.

Change in revision R05 compared to R04:

1. Primer mix 78 does not amplify the DRB1\*14:137N and 14:152N alleles. Primer mix 87 does not amplify the DRB1\*14:137N allele. This has been corrected in the Specificity and Interpretation Tables.

Change in revision R06 compared to R05:

1. Primer mix 51 does not amplify the B\*08:31, 41:06, 41:15 and 42:14 alleles, and primer mix 72 does not amplify the B\*08:31 allele. This has been corrected in the Specificity and Interpretation Tables.