



# Omixon Holotype HLA and Omixon HLA Twin

## Known product limitations

Version 18

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## 1 The scope of this document

The purpose of this document is to provide a comprehensive list of known product limitations for Holotype HLA and Omixon HLA Twin. The current version of this document was assembled using:

- Holotype HLA versions 2.1 (RUO&CE), 2.2 (RUO), 3.0 (RUO) and 3.0.1 (CE&RUO), and
- Omixon HLA Twin versions 4.1.0 (CE&RUO), 4.2.0 (CE&RUO), 4.2.2 (CE&RUO), 4.3.0 (RUO), and 4.4.0 (CE&RUO) with
- IMGT/HLA versions 3.39.0\_9, 3.40.0\_9, 3.41.2\_9, and 3.42.0\_9

Unless otherwise specified, the listed limitations are affecting all assay, software and database versions within the scope of this document.

For an overview of previous versions and changes of this document see the "Revision and change history" section.

## 2 Overview of generic product limitations

### 2.1 Holotype HLA-specific limitations

#### 2.1.1 False positive results affecting DRB3/DRB4 or DRB5

False positive results may be observed on very rare occasions for HLA-DRB3, HLA-DRB4 or for HLA-DRB5 with assay workflow version 3.0 and 3.0.1. The root cause of this sporadically occurring phenomenon is under investigation.

#### 2.1.2 Holotype HLA assay protocol version 3.0 specific limitation

When using the Holotype HLA v3.0 protocol some users may experience the presence of a white precipitate after the adaptor ligation step. We have found that this precipitate is formed by a cross-reaction of a compound in the Promega LR-PCR mix and one in the End Repair buffer. In some cases this precipitate may have small effects in the final library, but it does not affect the genotyping results. For advice on how to handle this phenomenon please contact [support@omixon.com](mailto:support@omixon.com) or your Field Application Scientist directly.

#### 2.1.3 Holotype HLA specific ambiguities

This section contains ambiguities which are caused by the design of the Omixon Holotype HLA assay and technological limitations of NGS (i.e., the location and sequence of primer sites and the fragment size distribution produced by the size selection method used in the protocol).

A multiple sequence alignment was created for each loci containing all allele sequences and the Holotype primer sequences. Then this alignment was trimmed to the targeted region (i.e. the primer sites and any position outside the primer sites were trimmed). The resulting sequences were then checked for exact duplicates and subsequence relations and all ambiguities on three field or lower resolution or at any resolution but affecting alleles with non-standard expression levels were collected.

#### 2.1.4 First, second and third field ambiguities

**Guidelines for Reporting:** Report as ambiguous



Ambiguous alleles		Affected IMGT/HLA version(s)	Level of ambiguity
DPB1*13:01:01	DPB1*107:01	All <sup>1</sup>	1st field
DPB1*105:01:01	DPB1*665:01	All <sup>1</sup>	1st field
DPB1*1072:01	DPB1*665:01	v3.40.0_9	1st field
DPB1*584:01:01	DPB1*584:01:02	All <sup>1</sup>	3rd field
DRB1*01:01:01	DRB1*01:100/ DRB1*01:01:35	All <sup>1</sup> / v3.42.0_9	2nd field/ 3rd field
DRB1*03:01:01	DRB1*03:147/ DRB1*03:01:31	All <sup>1</sup> / v3.42.0_9	2nd field/ 3rd field
DRB1*12:01:01	DRB1*12:10	All <sup>1</sup>	2nd field
DRB1*14:25:01	DRB1*14:25:02	All <sup>1</sup>	3rd field
DRB1*14:54:01	DRB1*14:216	All <sup>1</sup>	2nd field
DRB1*15:02:01	DRB1*15:140/ DRB1*15:149	All <sup>1</sup>	2nd field/ 2nd field
DRB1*15:03:01	DRB1*15:185	v3.41.2_9 v3.42.0_9	2nd field
DRB1*16:02:01	DRB1*16:64	v3.41.2_9 v3.42.0_9	2nd field
DRB3*01:62	DRB3*01:01:02:02	All <sup>1</sup>	2nd field
DRB3*02:02:01	DRB3*02:144	v3.42.0_9	2nd field
DRB4*01:03:01	DRB4*01:134	v3.42.0_9	2nd field

<sup>1</sup> All: All database versions within the scope of this document are affected.

## 2.1.5 Ambiguities affecting expression

**Guidelines for Reporting:** Low-expressing alleles are reported as 2nd field result

### Ambiguous allele groups

- HLA-A\*02:01:01:01/**02:01:01:02L**/02:01:01:16/02:01:01:50/02:01:01:145
- HLA-B\*39:01:01:03/**39:01:01:02L**/39:01:01:05/39:01:01:09/39:01:01:15
- HLA-DPA1\*02:02:02:NRS/**02:02:02:06Q**<sup>2</sup>
- HLA-DQB1\*03:01:03:19/03:29/03:191/03:297/03:312/03:377/03:419/03:431/**03:358N**<sup>1</sup>
- HLA-DRB1\*10:01:01/**10:38Q**

<sup>1</sup> Ambiguity is present with IMGT/HLA 3.36.0\_8 and above. HLA-DQB1\*03:358N contains a deletion in exon 3 which causes a frameshift and a premature stop in codon 191 (source: <http://hla.alleles.org/alleles/nulls.html>, date of access: 16-Jul-2019). As of 16-Jul-2019 this allele has been observed in two biological samples with unknown ethnic



origin by a single laboratory. No information was publicly available about the source sequences for this allele at the time this document was created. Based on the information available in IMGT/HLA 3.36.0, this allele cannot be distinguished from the other alleles listed in the ambiguous allele groups by the software, but it can be ruled out by manual comparison of the allele sequences in the gene browser. Note, that not all listed alleles are reported as ambiguous in all cases.

<sup>2</sup> HLA-DPA1\*02:02:02:06Q was added to IMGT/HLA 3.41.0. The allele sequences was found to be erroneous and was removed from the IMGT/HLA database in version 3.42.0.

## 2.1.6 Cis/Trans ambiguities

Cis/Trans ambiguities (i.e. ambiguous allele calls where the different allele pairs only differ in cis/trans phasing) can have multiple root causes. The majority of these ambiguities are reported due to limitations of the technology and the IMGT/HLA database.

**Guidelines for Reporting:** It is up to the individual lab whether to report the ambiguity using the G groups or to report the specific allele pairs that are ambiguous.

## 2.2 List of known limitations for Omixon HLA Twin

### 2.2.1 Known limitations of the Consensus Genotyping Algorithm

#### Introduction

All limitations listed below were based on observations reported by Omixon's customers or made during internal validation and regression testing.

#### False novelty called

Rarely, HLA Twin can report false novelties to the end user. Note, that the vast majority of these false novelties can be eliminated by manual inspection of the results in Omixon HLA Twin by a trained user.

#### Ambiguity is not reported for novel alleles

By design, only a single novel allele is reported by the Consensus Genotyping algorithm. In very rare cases, multiple equally probable novel alleles can be identified, but only one of these options is called by the algorithm.

#### Long novel indels missed

Rarely, long novel insertions or deletions are not reported by Omixon HLA Twin.

#### Novel allele missed (Fixed in Omixon HLA Twin 4.3.0)

In rare cases, novel alleles are missed on Omixon HLA Twin version 4.1.0, 4.2.0 and 4.2.2. This kind of novelties include cases with short indels or phasing issues.

#### Null allele called instead of novel allele

In rare cases, an unambiguous null allele is reported instead of a novel allele when the novel position falls within a deletion present in the null allele. Known cases affect the following alleles:

- HLA-DQB1\*03:276N/HLA-DQB1\*03:358N called instead of HLA-DQB1\*03:01:01:XX novel.

#### Incorrect phasing

In rare cases, the consensus sequences is phased incorrectly.



### Identifying incorrectly phased consensus sequences

Incorrect cis/trans phasing can be suspected if one or more of the following characteristics are observed:

- Two novel alleles are reported within a single best match pair.
- One novel allele and one partially defined allele is reported.
- One or two rare alleles are reported.
- There are several novel positions.

If incorrect phasing is suspected, the user is advised to inspect the results of the statistical genotyping algorithm.

### Cis/trans ambiguity due to inefficient phasing

In some rare cases, second or third field level ambiguities are reported due to inefficient phasing. In these cases, reanalysis of the affected loci with more reads is suggested.

### Ambiguity not reported (Fixed in Omixon HLA Twin 4.3.0)

In some rare cases:

- G-group level cis/trans ambiguities are not always reported for HLA-DPB1.
- The following common ambiguities are not always reported (reported alleles/**not reported alleles**):
  - HLA-DRB1 - DRB1\*12:10/ **DRB1\*12:01:01**, DRB1\*15:140/DRB1\*15:149/ **DRB1\*15:02**, DRB1\*03:147/**DRB1\*03:01:01**,
  - HLA-DQB1- DQB1\*03:276N/**DQB1\*03:01**.

### No result reported despite successful consensus generation (Fixed in Omixon HLA Twin 4.3.0)

In some cases, no allele call is reported even though a consensus sequence was successfully generated.

### Analysis cannot be completed with more recent IMGT/HLA database versions (Fixed in Omixon HLA Twin 4.2.0)

In some samples, genotyping with Omixon HLA Twin cannot be completed with IMGT/HLA 3.38.0 and above as the software runs out of memory. Very rarely, a similar phenomenon can be observed with earlier IMGT/HLA versions (e.g., version 3.37.0\_8).

### No result reported for some loci with IMGT/HLA 3.40.0 and above (Fixed in Omixon HLA Twin 4.4.0)

Frequently, no genotyping result is reported for HLA-A and less frequently for other loci with database versions IMGT/HLA 3.40.0 and above in combination with Omixon HLA Twin versions below version 4.4.0. The usage of the affected database versions in combination with any software version below version 4.4.0 is strongly discouraged and is officially not supported.

### The gene browser does not open for some novelties (Fixed in Omixon HLA Twin 4.4.1)

In some cases, the gene browser does not open and a data source error is shown for novelties in Omixon HLA Twin version 4.4.0.

### Incorrect alignment shown in the Gene Browser

In some rare cases where the reported alleles have significant length differences in some gene regions, the sequence tracks in the gene browser can be incorrectly aligned, and unnecessary gaps are shown. This issue does



not affect the allele call or the values calculated for QC metrics. Displaying alleles separately can eliminate this visualization defect.

Automated analysis is not functional in Omixon HLA Twin 4.4.0 (Fixed in Omixon HLA Twin 4.4.1)

Automated genotyping is not functional in Omixon HLA Twin version 4.4.0.

## 2.2.2 Known limitations of the Statistical Genotyping Algorithm

Due to the high similarity of the exon sequences of some alleles, the statistical genotyping algorithm reports incorrect alleles in some cases.

## 3 Known product limitations for HLA-B

### 3.1 Holotype HLA-specific limitations

#### 3.1.1 Alleles that may display low amplification

Low amplification means that the generated read count for an allele is not sufficient for genotyping. In extreme cases, the allele might not be reported at all (dropout).

Low amplification alleles	Compensation in HLA Twin	Detection resolution
B*51:01:02	YES	YES

### 3.2 Omixon HLA Twin specific limitations

#### 3.2.1 Known limitations of the Consensus Genotyping algorithm

Incorrect consensus sequence due to inefficient cross-mapping detection

- In some rare cases ambiguous results are reported due to partial loss of consensus at the consensus start.
- Rarely, false novelties are reported due to an incorrect consensus sequence near the consensus start.

#### HLA-B\*15:01 miscalled

In some very rare cases, alleles belonging to the following allele group can be miscalled and amino-acid information can be incorrect due to inconsistencies in the database:

- HLA-B\*15:01:01:01,
- HLA-B\*15:01:01:02N,
- HLA-B\*15:NEW

#### 3.2.2 Known limitations of the Statistical Genotyping algorithm

Some HLA-B alleles are miscalled due to the presence of an identical exon sequence in HLA-C

A group of HLA-B alleles (several HLA-B\*44 alleles and HLA-B\*47:04) have an exon 2 sequence identical to HLA-C\*16:85. Due to this similarity, these alleles can be miscalled by the statistical genotyping algorithm.



## 4 Known product limitations for HLA-DQB1

### 4.1 Holotype HLA-specific limitations

#### 4.1.1 Alleles that may display low amplification

Low amplification alleles	Compensation in HLA Twin	Detection resolution
DQB1*03	YES	YES <sup>1</sup>

<sup>1</sup>Suggestion based on Linkage Disequilibrium (LD) with DQA1

#### 4.1.2 Alleles that are not amplified

DQB1\*03:276N – due to a long deletion covering the 5' amplification primer site this allele is not amplified.

## 5 Known product limitations for HLA-DRB1

### 5.1 Technological limitations

Moderate allelic imbalance can be observed for alleles with significantly longer sequences than the average (e.g., some HLA-DRB1\*04 alleles). In some rare cases, high allelic imbalance can be observed. Sporadically, an allele dropout may occur.

#### 5.1.1 Holotype HLA-specific limitations

##### Low amplification

Moderate to high allelic imbalance can be observed for HLA-DRB1\*07 alleles in some cases. Very rarely, allele dropouts can be expected.

## 6 Known product limitations for HLA-DRB4

### 6.1 Holotype HLA-specific limitations

#### 6.1.1 Alleles that may display low amplification

Low amplification means that the generated read count for an allele is not sufficient for genotyping. In extreme cases, the allele might not be reported at all (dropout). Low amplification and allele dropouts have frequently been observed for HLA-DRB4\*01:01. In rare cases, allele dropouts have been reported for HLA-DRB4\*01:03 alleles. In both cases, the presence of the allele is suggested based on linkage disequilibrium by Omixon HLA Twin.

#### 6.1.2 Other assay related limitations

##### False positive concentration measurements for HLA-DRB4

High amplicon concentrations can be observed in some samples even though:

- the individual does not have a copy of the HLA-DRB4 gene or
- the individual does have one or two copies of the HLA-DRB4 gene, but amplification was not successful.



## 6.2 Omixon HLA Twin specific limitations





### 6.2.1 Known limitations of the consensus genotyping algorithm

Ambiguity is not reported

Result called by Twin	Correct result
DRB4*01:01:01:01	DRB4*01:01:01:01/DRB4*03:01N



## 7 Revision and change history





Version	Approval date	Author	Summary of changes	Approved by
v1	 05 Jul 2017	Krisztina Rigó	Algorithmic limitations collected. Document merged with the Holotype HLA-specific limitation document.	Efthymia Melista, Zoltán Simon, Peter Meintjes, Gabriella Adlovits
v2	 31 Jan 2018	Krisztina Rigó	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA v3.28.0 and v3.29.0.1. The software limitation section was extended to match the following software versions: Twin 2.1.3, Twin 2.1.4 and Twin 2.5.0.	Efthymia Melista, Gergely Tölgyesi, Gabriella Adlovits
v3	 04 Jul 2018	Krisztina Rigó	Additional phasing related cases were added. A short guide added for identifying incorrect phasing. Limitations related to the IMGT/HLA database were updated to match IMGT/HLA v3.30.0. The software limitation section was extended to match the following software versions: Twin 2.5.1 and Twin 3.0.0.	Efthymia Melista, Gergely Tölgyesi, Gabriella Adlovits
v4	 19 Oct 2018	Krisztina Rigó	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA v3.31.0. The software limitation section was extended to match the following software versions: Twin 3.1.0 and Twin 3.1.1. Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: Omixon HLA Twin 2.1.3 and 2.1.4, IMGT/HLA 3.28.0_4. Specific examples were removed for issues where allele specificity could not be proven. Additional limitations were added for the Statistical Genotyping algorithm.	Márton Pogány, Gergely Tölgyesi, Gabriella Adlovits





Version	Approval date	Author	Summary of changes	Approved by
v5	 14 Jan 2019	Krisztina Rigó	<p>Limitations related to the IMGT/HLA database were updated to match IMGT/HLA v3.32.0. Information related to IMGT/HLA versions older than 12+1 months was removed. Affected version: IMGT/HLA 3.29.0.1_5.</p> <p>The assay limitation sections were extended with the following assay version: Holotype HLA v3.0.</p> <p>Formatting was changed in the "Ambiguities affecting expression" section and a new case was added.</p> <p>An additional case was added to the HLA-DPB1 "Cis/Trans ambiguities" section.</p> <p>Additional minor changes and updates.</p>	Efthymia Melista, Gergely Tölgyesi, Gabriella Adlovits
v6	 26 Mar 2019	Krisztina Rigó	<p>Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.32.0_7, 3.33.0_7 and 3.34.0_8.</p> <p>The software limitation section was extended to match the following software version: Twin 3.1.3.</p> <p>Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: Omixon HLA Twin 2.5.0, IMGT/HLA 3.30.0_5 and 3.31.0_5.</p> <p>Product versions affected by the DQB1*03:276N ambiguity have been corrected and updated.</p> <p>Specific examples were removed from the HLA-DPB1 Cis/Trans ambiguities section.</p> <p>Additional minor changes and updates.</p>	Efthymia Melista, Gergely Tölgyesi, Gabriella Adlovits
v7	 26 Apr 2019	Krisztina Rigó	<p>Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.35.0_8.</p> <p>A limitation of the statistical genotyping algorithm caused by identical region sequences in different loci has been updated.</p> <p>A limitation of the consensus genotyping algorithm related to novel indels has been updated.</p> <p>Section "First, second and third field ambiguities" was restructured.</p>	Efthymia Melista, Gergely Tölgyesi, Gabriella Adlovits

Version	Approval date	Author	Summary of changes	Approved by
v8	 19 Jul 2019	Krisztina Rigó	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.36.0_8. Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: Omixon HLA Twin 2.5.1, IMGT/HLA 3.32.0_5, 3.32.0_7. A limitation of the consensus genotyping algorithm affecting HLA-DRB3 was added.	Efthymia Melista, Gergely Tölgyesi, Gabriella Adlovits
v9	 08 Aug 2019	Krisztina Rigó	The software limitation section was extended to match the following software version: Omixon HLA Twin 4.0.0. Information related to software versions older than 12+1 months was removed. Affected version: Omixon HLA Twin 3.0.0. A limitation of the consensus genotyping algorithm affecting HLA-DRB1 was added.	Efthymia Melista, Gergely Tölgyesi, Beatrix Kosiba
v10	 16 Oct 2019	Krisztina Rigó	The software limitation section was extended to match the following software version: Omixon HLA Twin 4.0.1.	Efthymia Melista, Gergely Tölgyesi, Gabriella Adlovits
v11	 19 Nov 2019	Krisztina Rigó	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.37.0_8. Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: Omixon HLA Twin 3.1.0, IMGT/HLA 3.33.0_7. Limitations of the Statistical Genotyping algorithm and ambiguity related limitations of the Consensus Genotyping algorithm were restructured.	Efthymia Melista, Gergely Tölgyesi, Gabriella Adlovits
v12	 07 Jan 2020	Krisztina Rigó	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.38.0_8. The software limitation section was extended to match the following software version: Twin 4.1.0. Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.34.0_8.	Efthymia Melista, Adrienn Kocsis, Gabriella Adlovits



Version	Approval date	Author	Summary of changes	Approved by
v13	 26 Mar 2020	Gergely Tölgyesi, Krisztina Rigó	<p>Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.38.0_9. The software limitation section was extended to match the following software version: Twin 4.2.0.</p> <p>Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.35.0_8.</p> <p>A limitation of the consensus genotyping algorithm affecting recent IMGT/HLA versions was added. The assay limitation section was extended with sporadically occurring DRB3/4/5 specific false positive issue, and assay workflow 3.0 specific phenomenon of the "white precipitant" and its suggested handling.</p>	Efthymia Melista, Gabriella Adlovits, Elmar Schilling
v14	 01 Jul 2020	Gergely Tölgyesi, Krisztina Rigó	<p>Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.36.0_8, Omixon HLA Twin versions 3.1.1 and 3.1.3.</p> <p>Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.39.0_9. The software limitation section was extended to match the following software version: Twin 4.2.2.</p> <p>Information related to Holotype HLA v1 was removed as this assay version is no longer supported.</p>	Libor Kolesar, Nándor Varga, Gabriella Adlovits, Gergely Tölgyesi, Krisztina Rigó
v15	 07 Aug 2020	Krisztina Rigó	<p>The software limitation sections were extended to match the following software version: Twin 4.3.0.</p> <p>Some limitations affecting Twin versions 4.1.0, 4.2.0, and 4.2.2 were added.</p>	Libor Kolesar, Zoltán Simon, Gabriella Adlovits, Gergely Tölgyesi, Krisztina Rigó
v16	 05 Oct 2020	Krisztina Rigó	<p>Information related to software versions older than 12+1 months was removed. Affected version: Omixon HLA Twin 4.0.0.</p>	Libor Kolesar, Elmar Schilling, Gabriella Adlovits, Gergely Tölgyesi, Krisztina Rigó



Version	Approval date	Author	Summary of changes	Approved by
v17	 14 Jan 2021	Krisztina Rigó	<p>Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected version: Omixon HLA Twin 4.0.1, IMGT/HLA 3.37.0_8, 3.38.0_8, and 3.38.0_9.</p> <p>The software limitation sections were extended to match the following software version: Omixon HLA Twin 4.4.0 (CE&amp;RUO). Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.40.0_9, 3.41.2_9, and 3.42.0_9, including limited version compatibility with Omixon HLA Twin. A new limitation of the consensus genotyping algorithm was added affecting novel alleles where the novel position is present within a deletion in a null allele similar to the novel allele.</p>	Libor Kolesar, Ákos Botos, Elmar Schilling, Gabriella Adlovits, Krisztina Rigó
v18	 02 Mar 2021	Krisztina Rigó	<p>Information related to software versions older than 12+1 months was removed. Affected version: Omixon HLA Twin 4.1.0.</p> <p>The software limitation sections were extended to match the following software version: Omixon HLA Twin 4.4.1 (CE&amp;RUO). Two limitations affecting Omixon HLA Twin 4.4.0 were added (automated genotyping is not functional, gene browser throws an error for some novelties).</p>	Libor Kolesar, Ákos Botos, Gabriella Adlovits