DYNAL BIOTECH

510(k) SUMMARY

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: B K 0 6 0 1 2

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APPENDIX 1

510(k) Notification

Sections 1.0 to 9.0

1.0 Introduction

The HLA class II molecules of the human Major Histocompatibility Complex (MHC) are encoded on the short arm of human chromosome 6 in the HLA-D region[1,2]. These glycoproteins consist of an alpha and a beta chain associated as heterodimers on the cell surface of antigen-presenting cells such as B cells and macrophages.The HLA-D region contains several class II genes and has three subregions: HLA-DR, -DQ and -DP. Both the HLA-DQ and -DP regions contain one functional gene each for the alpha and beta chains. The HLA-DR subregion contains one functional gene for the alpha chain; the number of functional genes for the beta chain varies from one to two depending upon the Class II haplotype[9]. All individuals express a DRB molecule which appears to play an important role in both graft rejection and graft versus host disease.
With the exception of the DRA molecule, the genes encoding the functional class II molecules are highly polymorphic with virtually all of the variability localised to the second exon. Within this exon, the polymorphism is concentrated into discrete clusters which lie within a relatively conserved framework region. This exon encodes the amino-terminal extracellular domain, which functions as the antigen binding site for processed peptides.

2.0 In Vitro Diagnostic Product Name

Proprietary Names:	Dynal RELI™ SSO HLA-DRB1 Typing Kit For use as a manual assay or with the AutoRELI™ 48 instrument.
Common Name:	HLA-DRB1 Typing Kit
Classification Name:	Unknown

3.0 Establishment Registration Number

Device Establishment Registration Number: To be Issued

4.0 Device Classification

HLA Typing Reagents are not classified.

5.0 Compliance to Classification Requirements

No performance standards have been established for HLA typing reagents. Dynal Biotech intends to comply with any standards applicable to the product developed in the future.

6.0 Intended Use

This DNA based typing kit provides a low to medium resolution HLA-DRB1 typing result.
This RELI™ SSO HLA-DRB1 typing system utilises two elements to achieve the HLA-DRB1 result.
All samples are first tested using the HLA-DRB1 typing kit. Most samples require no further testing beyond this first element but some allele combinations can lead to an ambiguous result that requires further analysis.

The HLA-DRB1*03/11/13/14 typing kit is the second element of the system and is designed to resolve many of the ambiguous allele combinations present after the HLA-DRB1 test. It is not designed as a stand-alone test and should only be performed after analysis of the result obtained from the HLA-DRB1 typing test.

The results from this test must not be used as the sole determinant for making clinical decisions.

The assay can be performed manually and results analysed using the interpretation table provided. The assay can be automated using the Dynal AutoRELI™ 48 Instrument.

7.0 Device Description

The Dynal RELI™ SSO HLA-DRB1 & DRB1*03/11/13/14 Tests are based on three major processes: PCR target amplification, hybridisation of the amplified products to an array of immobilized sequence-specific oligonucleotide probes, and detection of the probe-bound amplified product by colour formation.

PCR Amplification Reaction

The PCR reagent mixture containing the DNA specimen is heated to 96°C, separating the double-stranded DNA and exposing the specific primer target sequences. As the mixture cools, the biotinylated primers anneal to their targets. The thermostable recombinant *Thermus aquaticus* (Taq) DNA polymerase in the presence of excess deoxynucleoside triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine and deoxyuridine (in place of deoxythymidine), extends the annealed primers along the target templates to produce a biotinylated DNA sequence termed an **amplicon**. This process is repeated for a number of cycles, each cycle effectively doubling the amount of target DNA. For this test, the required number of cycles has been determined to be 35, theoretically yielding more than a billion-fold amplification.

Hybridisation Reaction

After the PCR amplification process, the amplicons are chemically denatured to form single-stranded DNA, these are added to a nylon membrane which contains an array of immobilized, sequence-specific oligonucleotide (SSO) probes. The biotin-labelled amplicons then bind (hybridise) to those SSO probes that contain a complementary target sequence and thus are "captured" onto the membrane strip.

A stringent wash step after hybridisation ensures the specificity of the reaction and removes all unbound amplicon.

Detection Reaction

The amplicon- probe complex is visualised using a colourmetric reaction. Streptavidin-horseradish peroxidase (SA-HRP) conjugate is added to the membrane and binds to the biotin-labelled amplicons captured by the SSO probe. Addition of hydrogen peroxide (H_2O_2) and tetramethylbenzidine (TMB) substrate, results in the formation of a blue colour complex in the presence of SA-HRP. The resulting probe signals are compared to the control probe intensity and the samples hit pattern recorded for interpretation.

8.0 Proposed Labelling and Instructions for Use (IFU)

The proposed labels for Dynal RELI™ SSO HLA-DRB1 Typing Kit reagents are presented in Appendix 1. The proposed IFU for RELI™ SSO HLA-DRB1 Typing Kit appears in Appendix 2.

9.0 Statement of Substantial Equivalence

Summary of Substantial Equivalence

Dynal have already received 510(k) clearance for the following RELI™ SSO Typing Kit products:

HLA-A	BK030056
HLA-B	BK030057
HLA-Cw	BK030058
HLA-DQB1	BK030059
HLA-DRB	BK030060

Dynal is now submitting the new HLA-DRB1 kit.

HLA-DRB1 typing at low to medium resolution is routinely performed worldwide using DNA based technology. The method chosen to use as a predicate device to evaluate the performance of the RELI™ SSO typing system is based on the reverse SSO technology used by Dynal RELI™ SSO.

Dynal RELI™ HLA-DRB1 Typing System is substantially equivalent to the following predicate device

SSO: One Lambda Inc. LABType™ SSO (BK020055)

Comparison of Dynal RELI™ HLA-DRB1 & DRB1*3/11/13/14 Typing Kits to Predicate Device

The predicate device achieves a low to medium resolution HLA-DRB1 type with some differences in technology to Dynal RELI™ SSO. Dynal Biotech believes that RELI™ SSO maintains the same safety and effectiveness as the predicate device.

Comparison of Dynal RELI™ SSO DRB1 typing kit with the Predicate device One Lambda Inc. LABType™ SSO (BK020055)

	Dynal RELI™ SSO	One Lambda Inc. LABType™ SSO
Intended Use	Low to medium Resolution HLA-DRB1 typing result	Low to medium Resolution HLA-DRB1 typing result
Assay Methodology	Reverse Sequence Specific Oligonucleotide (SSO) Probe Detection	Reverse Sequence Specific Oligonucleotide (SSO) Probe Detection
Amplification	Primers Specific for Exon 2 of DRB1.	Primers Specific for Exon 2 of DRB1.
Probe array	Probes are immobilised on nylon membrane strip to form array.	Each probe is immobilised on an individual fluorescently coded microsphere and mixed to form array.
Specimen type	High quality DNA	High quality DNA
Detection method	Visual colourmetric reaction	Reaction intensity using flow analyser
Results assignment	Positive and negative probe assignment made manually in comparison to reference probe signal	Positive and negative probe assignment made by computer against intensity cut off values held in software
Results Interpretation	Manually using a Hybridisation Pattern Interpretation Table	Manually using a Hybridisation Pattern Interpretation Table

Summary of Specific Performance Characteristics

Dynal RELI™ SSO HLA-DRB1 Typing Kit was compared to the FDA approved method stated above. A total of 94 samples (188 allele assignments) were tested. There was 94.6% concordance (89/94) for HLA-DRB1 loci between the RELI™ SSO HLA-DRB1 typing system and the results obtained using the other method. 2 samples required the DRB1*03/11/13/14 typing kit to provide resolution at the allele group level. Further analysis of the 5 non-concordant results showed that other SSO methodology gave an ambiguous result for all 5 samples. In all 5 cases, one of the options contained within the ambiguous result matched the unambiguous result provided by RELI™ SSO.

HLA-DRB1 Allele groups

Allele Group	Allele Group	Allele Group
DRB1*01 (not 0103)	DRB1*09	DRB1*15
DRB1*0103	DRB1*10	DRB1*16
DRB1*03	DRB1*11	
DRB1*04	DRB1*12	
DRB1*07	DRB1*13	
DRB1*08	DRB1*14	

Conclusion

This study shows that Dynal RELI™ SSO HLA-DRB1 typing system provides low to medium resolution results that are reliable compared to existing FDA approved methodology.

A complete report of the performance study is provided in Appendix 5