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: _____

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Summary Report Date: 11th August 2003

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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. They play a central role in the regulation of the immune system[3,4] in transplantation biology[5], as well as in susceptibility to a number of diseases, including auto-immune disorders[6,7] and certain cancers[8]. 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-DRB Typing Kit Dynal RELI™ SSO HLA-DRB1*03/11/13/14 Typing Kit
Common Name:	HLA-DRB Typing Kit HLA-DRB1*03/11/13/14 Typing Kit
Classification Name:	Unknown

3.0 Establishment Registration Number

Device Establishment Registration Number: To be Issued

4.0 Device Classification

HLA Typing Reagents have been classified as Class I devices

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 system provides a low to medium resolution HLA-DRB1 & DRB3/4/5 typing result.
The Dynal RELI™ SSO HLA-DRB typing kit uses a generic amplification of DRB1, 3, 4 & 5 genes to provide a result at the allelic group level (see section 5.1) for the majority of DRB1 allele combinations.
For some DRB3 associated alleles (DRB1*03/11/13/14) the generic amplification does not resolve the DRB1 allele combination to the allele group level. This requires a second test to be performed on the sample using Dynal RELI™ SSO HLA-DRB1*03/11/13/14 typing kit. This is not a stand alone test and should be performed only after analysis of the result obtained from the HLA-DRB Typing test.

7.0 Device Description

The Dynal RELI™ SSO HLA-DRB & 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 95°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-DRB & HLA-DRB1*03/11/13/14 Typing Kit reagents are presented in Appendix A. The proposed IFU for RELI™ SSO HLA-DRB Typing Kit appears in Appendix B. The proposed IFU for RELI™ SSO HLA-DRB1*03/13/14 typing Kit appears in Appendix C.

9.0 Statement of Substantial Equivalence

Summary of Substantial Equivalence

Dynal RELI™ SSO HLA-DRB Typing System provides a low to medium resolution HLA-DRB typing result. It has been on sale for Research Only purposes since June 1999. Dynal Biotech now wishes to broaden the scope of this assay for clinical testing by gaining FDA clearance via this submission.

HLA-DRB typing at low to medium resolution is routinely performed worldwide using DNA based technology. No single method was chosen to use as a predictive device to evaluate the performance of the RELI™ SSO typing system but the chosen devices are both based on the reverse SSO technology used by Dynal RELI™ SSO.

Dynal RELI™ HLA-DRB Typing System is substantially equivalent to the following predictive devices

SSO: One Lambda Inc. LABType™ SSO (BK020055)
Biotest Diagnostics DQB1 SSO-Typing System (BK950015)

Comparison of Dynal RELI™ HLA-DRB & DRB18093/11/13/14 Typing Kits to Predictive Devices

Both predictive devices achieve a low to medium resolution HLA-DRB type with some differences in technology to Dynal RELI™ SSO. Dynal Biotech believes that RELI™ SSO maintains the same safety and effectiveness as the predictive devices.

RELI™ SSO:

The methodology is described in detail in section 2.0

Labtype SSO:

The predictive device utilises exactly the same reverse SSO principle as RELI™ SSO with the following modifications to the methodology

1. Each probe in the array is immobilised on an individual fluorescently coded microsphere rather than a membrane support.
2. Probe signals are detected using a flow analyser rather than visually using a colourmetric reaction.

Biotest SSO:

The predictive device utilises exactly the same reverse SSO principle as RELI™ SSO with the following modifications to the methodology

1. Each probe in the array is immobilised onto the well of an Elisa plate rather than a membrane support.