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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1.0 Introduction

The HLA loci of the human Major Histocompatibility Complex (MHC) located on the short arm of human chromosome 6 encode two distinct classes of highly polymorphic cell surface molecules that bind and present processed antigens in the form of peptides to T lymphocytes. This presentation step is crucial in initiating both cellular and humoral immune responses; consequently these molecules play a central role in the regulation of the immune system, in transplantation biology, as well as in susceptibility to a number of diseases, including auto-immune disordersand certain cancers.
The class I molecules, HLA-A, HLA-B, and HLA-C, are found on most nucleated cells. They are cell surface glycoprotein's that bind and present processed peptides derived from endogenously synthesized proteins (e.g., viral and tumour peptides) to $CD8^+$ T cells. These heterodimers consist of an HLA-encoded alpha chain associated with the non-MHC-encoded polypeptide, β2-microglobulin. While β2-microglobulin is monomorphic, the alpha-chain genes are extremely polymorphic. This variability is localized primarily in exons 2 and 3, which encode the amino-terminal extracellular domains that function as the peptide-binding site. Within these two exons, the polymorphism is concentrated into discrete clusters that lie within a relatively conserved framework region. Analysis of HLA class I crystal structures, has shown that these polymorphic residues line the peptide-binding cleft and interact directly with peptide and/or the T-cell receptor.

2.0 In Vitro Diagnostic Product Name

Proprietary Name: Dynal RELI™ SSO HLA-Cw Typing Kit

Common Name: HLA-Cw 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

Dynal RELI™ SSO HLA-Cw typing kit provides a low to medium resolution HLA-Cw typing result.

7.0 Device Description

The Dynal RELI™ SSO HLA-Cw Test is 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-Cw Typing Kit reagents are presented in Appendix A. The proposed IFU appears in Appendix B.

9.0 Statement of Substantial Equivalence

Summary of Substantial Equivalence

Dynal RELI™ SSO HLA-Cw Typing Kit provides a low to medium resolution HLA-Cw 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-Cw 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, as this reflects the diversity of commercial options available to customers and allows more customers to correlate performance data with the typing system they are currently performing.

Dynal RELI™ HLA-Cw Typing Kit is substantially equivalent to the following predictive devices

SSO:	One Lambda Inc. LABType™ SSO (BK020055)
SSP:	One Lambda Inc. MicroSSP™ (BK960062) & Pel Freez SSP UniTray® (BK000019)

Comparison of Dynal RELI™ HLA-Cw Typing Kit to Predictive Devices

All three predictive devices achieve a low to medium resolution HLA-Cw 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