9. HLA Diagnostics

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Visit www.dynalbiotech.com for the latest product up-dates and references.
HLA Diagnostics

Tissue Typing by Dynal RELI™ SSO – HLA Class I & II Strips
The Dynal RELI™ SSO product line for tissue typing was developed in our purpose-built manufacturing facility in the U.K. The Dynal RELI™ SSO technique utilises a generic PCR amplification, with biotinylated primers flanking the specific locus, followed by hybridisation of the resulting amplicon to an array of Sequence Specific Oligonucleotide (SSO) probes immobilised on a nylon membrane. The SSOs are designed to hybridise with the polymorphic sequences in the target HLA locus.

Under appropriate hybridisation and wash conditions, these probes will bind only to their complementary sequence in the amplified DNA and are able to distinguish single nucleotide differences. The presence of PCR product bound to a specific probe is detected using a simple colorimetric reaction and appears as a blue precipitate. The immobilisation of the SSOs is referred to as a “Reverse blot”; this and the “Line blot” pattern of the SSOs give rise to the acronym ‘RELI™’.

Interpretation of the HLA type is based on the pattern of positive probes and can be done using Dynal PMP computer software.

The RELI™ system is now fully automated. The AutoRELI™ 48 instrument performs the washing and hybridisation steps of the RELI™ SSO assay. The AutoRELI™ tray containing processed strips is transferred to the new RELI-Scan™ instrument. Strip images are scanned directly from the tray into our PMP software for immediate result interpretation. This saves time and increases throughput by eliminating manual handling of strips.

Tissue Typing by Dynal SSP
The Dynal SSP method is a PCR-based technique, which uses Sequence Specific Primers (SSP), for DNA-based HLA tissue typing.

Dynal SSP is based on the principle that a perfectly matched primer will be more efficiently used in the PCR reaction than a primer with one or several mismatches in its 3’-end.

The specificity of the typing system is part of the PCR amplification step, and the post-amplification processing of the samples is reduced to a minimum.

The assignment of alleles merely consists of determining whether amplification has occurred or not, i.e. visualisation and detection of the amplification by agarose gel electrophoresis. The typing is done in less than 2.5 hours.

Specially designed software can then be used to interpret results quickly and accurately.
HLA Dynabeads® for Cell Isolation
Dynabeads® HLA Class I/Cell Prep I and Dynabeads® HLA Class II/Cell Prep II are designed specifically for the rapid isolation of peripheral blood lymphocytes. This effective system is ideal for difficult patient samples (e.g. uraemic, leukaemia, cadaver donors) and offers high sensitivity and clear-cut interpretation. The isolated cells are suitable for multiple downstream applications and are the perfect partners for our serological product range.

Serological Typing
Serologic techniques can be used for HLA typing, antibody screening and cross-matching. The Pel-Freez brand serologic products are based on the microlymphocytoxicity assay. In this procedure, isolated target lymphocytes are incubated with human or monoclonal serum containing antibodies directed against HLA antigens found on the lymphocyte surface. The cell-serum mixture is combined with rabbit serum. The presence of an antigen-antibody complex "fixes" the complement, which results in breakdown of the cell membrane. Cells that do not express the antigens against which the antibody is directed will not fix complement and will remain intact. The breakdown of the cell membrane is indicated by uptake of a fluorescent label or vital dye. The product offering includes trays for HLA typing and antibody screening, along with rabbit complement, pooled human serum, antisera and control sera.

Sequence-Based Typing using SeCore™
SeCore™ HLA DNA typing can be used to achieve high resolution, gold standard typing of sample genomic DNA for bone marrow transplant. These sequencing products definitively identify DNA sequences from target HLA Class I and Class II genes. The sample genomic DNA is cycled with a locus specific amplification mix and FastStart™ Taq Polymerase. The resulting product is treated with ExoSAP-IT™, prior to sequencing, to degrade the unincorporated primers and hydrolyse the free nucleotides. The nucleotide chronology, and resulting HLA subtype, is determined by multicolour, fluorescence-based, DYEnamic™ ET (Thermosequenase II) terminator sequencing. The final reactions are purified, denatured and loaded on an automated ABI PRISM sequencing instrument.

Dynaplex™ - Microbead Reverse SSO Typing on Luminex 100™ System
Dynaplex™ HLA DNA typing can be used to achieve low resolution typing of sample genomic DNA. This is a high throughput method capable of typing from one to hundreds of samples a day. The Dynaplex™ HLA DNA typing method is a liquid bead-based reverse SSOP (Sequence Specific Oligonucleotide Probe) method. A biotinylated locus specific amplicon is generated from the sample genomic DNA by PCR and subsequently denatured. This product is combined with a single cocktail of colour-coded polystyrene beads. Each bead colour presents a unique group specific oligonucleotide probe. The beads presenting complementary probes to the amplicon will hybridise. Amplicons annealed to the conjugated probes are detected via SAPE (Streptavidin Phycoerythrin) chemistry. This chemical tag, bound to the biotinylated amplicon, is excited by one of the two lasers on the Luminex 100™ flow-based instrument. The second laser identifies the associated bead colour. The combined data is interpreted by Dynal's proprietary software, MatchPro™, identifying positive signal beads and their respective colour for allele group assignment.
Antibody Analysis using DynaChip™ Technology

The DynaChip™ Antibody Analysis product is an ELISA-based microarray chip assay in which HLA antigens are bound to the surface of the chip. Presented in a 96-well ELISA-type frame, each well contains a single test.

DynaChip™ technology is a unique approach to antibody analysis which combines the ease and familiarity of ELISA with the innovation and convenience of microarray technology, offering high throughput and fully automated assay processing.

The DynaCHIP™ Processor is our specially designed instrumentation for DynaChip™ assay processing. This offers a completely automated assay processing, from serum sample input to results interpretation output, with no manual handling of the chips.

**Tissue Typing from HLA Diagnostics**

**Sample**
- DNA Extraction

**Serology**
- Dynabeads® / Serology Products
  - FEATURES:
    - Simple, effective and reliable
    - Cost effective technique
    - Excellent supplement for other molecular techniques

**SSP**
- AllSet™ / UniTray™ SSP Typing
  - FEATURES:
    - Extensive product line
    - Easy technique and interpretation
    - Rapid – suitable for on-call typing
    - Frequently updated for new alleles
    - Superior technical and customer service

**SSOP**
- RELI™ SSO Typing
  - FEATURES:
    - Intermediate resolution
    - High throughput
    - Simple protocol and quick data acquisition and analysis
    - Stable hybridisation signal
    - Minimal hands-on time
    - Consistent and reproducible results, targeting the most polymorphic exons

**Sequence-Based Typing**
- SeCore™
  - FEATURES:
    - Gold standard for high resolution HLA typing
    - Identical cycling profiles for all loci
    - Utilises DYEnamic ET (Energy Transfer) Terminator Chemistry
    - Reliable bidirectional sequencing of the most polymorphic exons

**Microbead Reverse SSO Typing on Luminex 100™ Using DynaFlex™**
- FEATURES:
  - High throughput
  - Simple protocol and quick data acquisition and analysis
  - Stable hybridisation signal
  - Minimal hands-on time
  - Consistent and reproducible results, targeting the most polymorphic exons

**Compatible**
- With multi-dye capillary systems
- With existing sequencing software

**AutoRELI™ EL**
- Automated strip hybridisation and detection

**RELIScope™**
- Scans typing strip results directly from the AutoRELI™ tray

**SSO Typing Software**
- Specially designed interpretation software for RELI™ SSO

**Ordering Information**
For detailed ordering information for the HLA Diagnostic product range, please go to [www.dynalbiotech.com](http://www.dynalbiotech.com)