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SBT HLA-DQA1 High Resolution Typing Procedure

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High resolution typing of HLA-DQA1 is performed using group specific PCR amplification of exon 2 followed by sequenced based typing (SBT). SBT is performed by automated fluorescent sequencing with the ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Genomic DNA is first extracted from frozen whole blood, PBLs, granulocytes or B-cell lines using the QIA-AMP kits (QIAgen, Valencia, CA). The concentration and purity of DNA is determined by spectrophotometry and the working concentration adjusted to 20 ng/µL.

HLA-DQA1*02, 04, 05 and 06 alleles have a deletion at codon 56 which causes a 3 nucleotide shift in the electropherograms of samples heterozygous for HLA-DQA1*02, 04, 05, or 06 in combination with HLA-DQA1*01 or *03. To avoid this problem, we perform group specific amplification of HLA-DQA1 alleles followed by SBT. Thus, primers were designed to amplify exon 2 of HLA-DQA1 at group specific sites flanking the regions of allelic variation. All PCR reactions are checked to confirm the presence of amplified products of the expected size prior to sequencing.

PCR products are purified using ExoSAP-IT (USB, Cleveland, OH) treatment to remove unincorporated primers and dNTPs and then used as a template for direct sequencing.

Bi-directional sequencing is performed using DYEnamic ET terminator chemistry on the capillary-based ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and assignment of HLA-DQA1 alleles is made.

The above protocol was designed, via the use of group specific amplification of HLA-DQA1 alleles, to avoid the problem of ambiguous allele combinations. However, in the event an ambiguous allele combination is questioned, sequence specific primer (SSP) and/or sequence specific oligonucleotide probe (SSOP) methodology is used to confirm results.