DETECTION OF CLONAL T-CELL RECEPTOR-β (TCR β) GENE REARRANGEMENTS IN T-CELL MALIGNANCIES

Ampli TCRβ Cat. n.1.400.3

The T cell receptor (TCR) is a membrane bound heterodimer composed of two polypeptide chains (αβ or γδ) linked by a disulfide bond and associated with a non-polymorphic cytoplasmic membrane bound complex of proteins collectively known as CD3. In the peripheral blood, most T cells express the αβ receptor and up to 15% express the γδ receptor. The extracellular domains of the TCR consist of a constant (C) domain and a variable (V) domain. Hypervariable regions or complementarity determining regions (CDR) encoded within the TCR V domains contact the peptide antigen and the major histocompatibility (MHC) molecules and therefore confer the specificity of the TCR.

The genes encoding the TCRβ (TCRB) chain are located on the long arm of chromosome 7, at band 7q34. The TCRB locus contains approximately 67 V genes of which 47 are functional and 19 are pseudogenes. The functional TCRBV genes are grouped into 23 families based upon greater than 75% nucleotide sequence identity. TCRBV genes are located upstream of two D–J–C clusters. Both C gene segments (C1 and C2) are preceded by a D gene (D1 and D2) and a J cluster, which comprises six (J1.1–J1.6) and seven (J2.1–J2.7) functional J segments.

During early T-cell development, the rearrangement of the TCRB gene consists of two consecutive steps: D to J rearrangement and V to D–J rearrangement. Owing to the presence of two consecutive TCRB D–J clusters, it is also possible that two rearrangements are detectable on one allele: an incomplete TCRB D2–J2 rearrangement in addition to a complete or incomplete rearrangement in the TCRB D1–J1 region. In TCRB gene rearrangements, a non-random distribution of gene segment usage is seen. In healthy individuals, some V families predominate in the PB T-cell repertoire (eg V1–V5), while others are only rarely used (eg V11, V16, V18, and V23). The representation of J segments is also far from even. The J2 family is used more frequently than the J1 family (72 vs 28% of TCRB rearrangements).

Molecular analysis of the TCRB genes is an important tool for the assessment of clonality in suspect T-cell proliferations. In normal and reactively proliferating T cells, these genes are rearranged differently (ie, polyclonal), whereas in T-cell malignancies, the neoplastic cells contain identically rearranged monoclonal TCR genes. TCRB gene rearrangements occur not only in almost all mature T-cell malignancies, but also in about 80% of the CD3¯T-cell ALLs (T-ALL) and 95% of the CD3+T-ALL. TCRB rearrangements are not restricted to T-lineage malignancies as about one-third of precursor B-ALL harbor rearranged TCRB genes. Their frequency is much lower (0–7%) in mature B-cell proliferations.

The **AMPLI TCRβ** is a three-tube TCRB multiplex PCR system for clonality assessment in suspect T-cell proliferations with an high clonality detection rate according to the standardized BIOMED-2 PCR protocol (JJM van Dongen et al. Leukemia 2003).

Principle of assay: a) extraction of genomic DNA; b) multiplex PCR; c) detection in polyacrylamide gel by Heteroduplex analysis.
Applicability: on extracted and purified genomic DNA from whole blood samples.
Number of tests: 50

REAGENTS AND STORAGE

AMPLIFICATION	
Mix PCR TCRB-A	-20°C
Mix PCR TCRB-B	-20°C
Mix PCR TCRB-C	-20°C
H_2O RNase/DNase-free	-20°C
Taq Polymerase (5U/µl)	-20°C
Control DNA	-20°C

Stability: over 18 months if correctly stored.

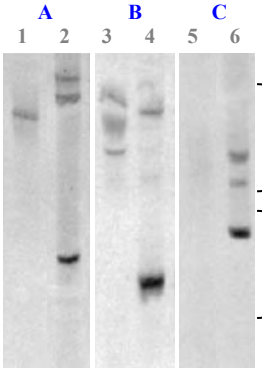
References:
van Dongen JJM et al. Clin Chim Acta, 1991
Arden B et al. Immunogenetics, 1995
Langerak AW et al. Leukemia, 1999
Szczepanski T et al. Leukemia, 1999
van Dongen JJM et al. Leukemia, 2003

ANALYSIS OF RESULTS

Heteroduplex analysis of PCR products: PCR products are denatured followed by rapid random renaturation at low temperature. This enforced duplex formation results in many different heteroduplexes with different migration speed in case of polyclonal lymphoproliferations, but results in homoduplexes with identical rapid migration in case of monoclonal lymphoproliferations. Electrophoresis of the homoduplexes in a 6% polyacrylamide gel results in a single band within a predictable size range, whereas the heteroduplexes form a smear at a higher position.

Expected size range

- TCRB tube A Vβ-Jβ	240–285 bp
- TCRB tube B Vβ-Jβ	240–285 bp
- TCRB tube C Dβ-Jβ	285–325 bp (Dβ1) 170–210 bp (Dβ2)



POLYCLONAL
samples: 1, 3 and 5
Heteroduplexes (HE) form a smear at a higher position

MONOCLONAL
samples: 2, 4 and 6
Homoduplexes (HO) results in a single band within a predictable size range