



Spoligotyping Technical Description

SPOLIGOTYPING is a PCR-Based Method to Simultaneously Detect and Type H37RV and BCG P3. The typing method is based on DNA polymorphism present at one particular chromosomal locus, the "Direct Repeat" (DR) region, which is uniquely present in H37Rv and BCGP3. The presence or absence of the strains can be detected by hybridization of PCR-amplified sample to a set of immobilized oligonucleotides on the membranes. Spoligotyping is similar to Southern blotting when rapid results are required. By Spoligotyping, one can detect the presence or absence of the spacers by the known sequence.

Oligonucleotides derived from the known spacers in the DR cluster are covalently linked to an activated membrane in parallel lines. PCR products are hybridized perpendicular to the oligo lines. After hybridization the membrane is incubated in streptavidin peroxidase, which binds to the biotin label on the PCR products. Detection of hybridization signals is optimized by the enhanced chemiluminescence (ECL) detection system (of course any biotin-detection method can be used when optimized). The peroxidase present on the streptavidine catalyzes a reaction resulting in the emission of light which can be detected by autoradiography of the membrane.

Kit Contents (IM9701) This kit for spoligotyping consists of four vials, one membrane and one manual.

- 1) Two vials containing the positive controls: a) Positive control 1 (Type H37Rv) b) Positive control 2 (BCG P3) Both positive controls are 10ng/ μ l. For PCR amplification, 2 μ l of each positive control should be added to the PCR mixture.
- 2) Two vials containing the primers for PCR amplification: a) Primer DRa (biotinylated) b) Primer DRb The primers are shipped lyophilized. To prepare both primers for PCR amplification, both primers should be dissolved in 1.72 ml MQ water.
- 3) The volume in each vial should be sufficient for approximately 430 PCR's.
- 4) One Spoligo-membrane The membrane is shipped in 20 mM EDTA.

Storage condition on Receipt

- 5) Store the membrane at + 4°C for optimal storage condition.