분류번호	II-P-6

Cloning, Expression and Purification of HIV-1 Reverse Transcriptase

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Virus-encoded HIV-1 reverse transcriptase (RTase) is one of the major targets for the development of drugs for HIV-1 since it is an essential enzyme for the replication cycle of HIV-1. We cloned the entire reverse trancriptase gene into an inducible expression vector with tac promotor. RTase was stably overexpressed and induced by IPTG and the highly-expressed RTase was purified partially by use of DEAE cellulose and Mono Q column. The partially purified enzyme (66kDa, 51kDa) as exhibited by SDS-PAGE showed the high specific activity (16,570U/mg) when the assay for the RTase activity was carried out using ^{3}H-dTTP and poly(rA):oligo(dT)$_{12-18}$ as the substrate.