



HIV-1 Reverse Transcriptase, Functional

HIV-1 reverse transcriptase is an RNA-dependent DNA polymerase derived from HIV-1 (AIDS virus), subtype B origin (Ref.1). It also has RNaseH activity and is an enzyme indispensable for reproduction of AIDS virus.

This protein is uniquely over-expressed as a recombinant protein in *E. coli* by a patented method and highly purified (Ref.2). It is composed of two subunits whose molecular weights are 66 kD and 51 kD, same as the those of the enzyme purified from AIDS virus particles (Fig 1).

Applications

- 1) It is extremely effective for screening new specific inhibitors for HIV virus as a drug for treating AIDS (Ref.3).
- 2) Generally, Gag and Env proteins are employed as antigens for detecting anti-HIV-1 antibody. However, by using this enzyme in combination as an antigen, the detection will be more sensitive.
- 3) Reverse transcriptases are used in the first step of RT-PCR reaction for converting RNA to DNA. The HIV-1 reverse transcriptase can also be applied for RT-PCR method.
- 4) Standards for SDS-PAGE (Fig.1), Western blotting (Fig.2), Dot blotting, ELISA

Definition of activity: Activity of intake of 1 nmole of dTMP in 10 min at 37°C is considered as 1 unit using poly(rA) and oligo(dT) as template and primer.

Conditions of measurement: 50 mM Tris-HCl (pH 8.3), 10 mM MgCl₂, 50mM KCl, 3 mM DTT, 0.1% Nonidet P-40, 20 ug/ml poly(rA) · oligo(dT)₁₂₋₁₈, 0.5 mM dTTP ([³H]dTTP, ~1 × 10⁵ cpm), and 10-50 units/ml reverse transcriptase.

Purity: Over 90% by SDS-PAGE (CBB staining)

Protein concentration: 0.4 mg/ml as measured by BCA method

Activity: 4,000 units/ml

Form: 50% glycerol, 40 mM Tris-HCl (pH8.3), 50 mM NaCl, 5 mM MgCl₂, 0.1% Triton X-100, 1 mM DTT

Size: 200 unit

Storage: -20°C

Data Link GenBank: [AAA44988.1](https://www.ncbi.nlm.nih.gov/nuccore/AAA44988.1)



References

1. Adachi A *et al* "Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone." *J Virol* **59**: 284-291 (1986) PMID: [3016298](#)
2. Saitoh A *et al*. "Overproduction of human immunodeficiency virus type I reverse transcriptase in Escherichia coli and purification of the enzyme." *Microbiol Immunol* **34**: 509-521 (1990) PMID: [1699113](#)
3. Fischl MA *et al* "The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo- controlled trial." *N Engl J Med* **317**: 185-191 (1987) PMID: [329089](#)

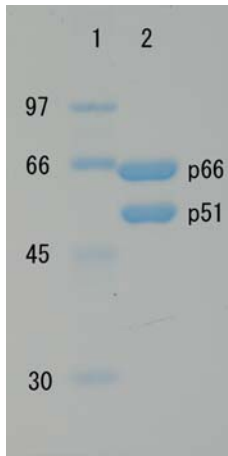


Fig.1 Polyacrylamide gel electrophoresis of HIV-1 reverse transcriptase protein

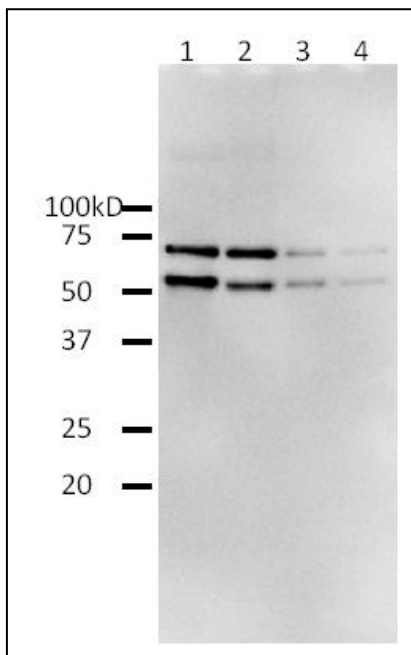


Fig.2. Western blotting of functional recombinant full-length HIV-1 reverse transcriptase by using anti-HIV-1 Reverse Transcriptase antibody (#65-001).

- 1; 40 ng / lane
- 2; 20 ng / lane
- 3; 4 ng / lane
- 4; 2 ng /lane

Anti-HIV-1 RT antibody was used at 1/2,000 dilution. As second antibody, goat anti-rabbit IgG antibody conjugated with HRP was used at 1/5,000 dilution. ECL system was used.

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