HIV-1 Reverse Transcriptase, Functional

05-001 200 units, 05-002 1000 units

Shipping and Storage: Ship at 4℃ or-20℃ and store at -20℃. Do not freezebelow -20℃

Product: Recombinant full-size HIV-1 Reverse Transcriptase without Tag-peptide expressed in E. coli. It is composed of heterodeimer, p66 and p51 as the one produced in HIV-1 infected cell. It 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.

Applications

1) It is extremely effective for screening new specific inhibitors for HIV virus as a drug for treating AIDS).
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) 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℃ 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 $MgCl_2$, 50mM KCl, 3 mM DTT, 0.1% Nonidet P-40, 20 ug/ml poly(rA) ・ $oligo(dT)_{12\text{-}18}$, 0.5 mM dTTP ($[^3H]$dTTP ,~1 x 10^5 cpm), and 10-50 units/ml reverse transcriptase.

Activity: ~5,000 units/ml

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

Form: 0.5 mg/ml in 50% glycerol, 40 mM Tris-HCl (pH8.3), 50 mM NaCl, 5 mM $MgCl_2$, 0.1% Triton X-100, 1 mM DTT

Data Link : UniProtKB: P04585 GenBank: AAA44988.1

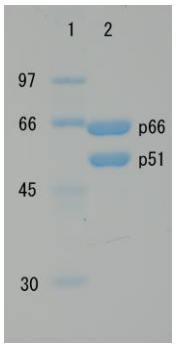

Fig. SDS-Polyacrylamide gel electrophoresis of HIV-1 reverse transcriptase

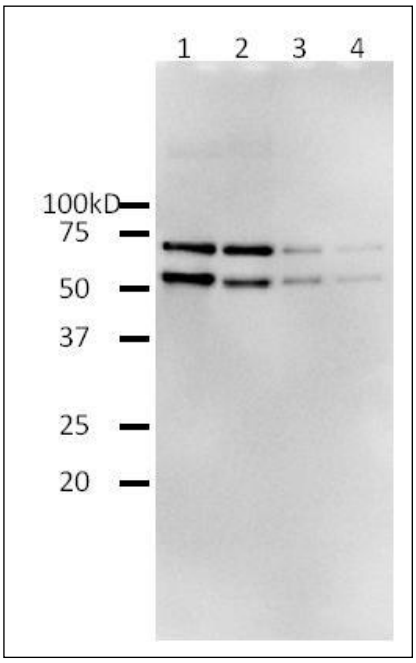

Fig.2. Western blotting of functional recombinant full-length HIV-1 reverse transcriptase by using anti-HIV-1 Reverse Transriptase antibody (BioAcademia 65-001).

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.

References: This product was described in Ref 1 and used in the following publications.

1. 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
2. Permanasari ED et al. Enzymatic Activities of RNase H Domains of HIV-1 Reverse Transcriptase with Substrate Binding Domains of Bacterial RNases H1 and H2. Mol Biotechnol. 2015 Jun;57(6):526-38. PMID: 25673083
3. Kadokura K et al. Novel urushiols with human immunodeficiency virus type 1 reverse transcriptase inhibitory activity from the leaves of Rhus verniciflua. J Nat Med. 2015 Jan;69(1):148-53. PMID: 25349048
4. Tada K et al. Abacavir, an anti-HIV-1 drug, targets TDP1-deficient adult T cell leukemia. Sci Adv. 2015 Apr 24;1(3):e1400203. PMID: 26601161
5. Izumida M et al. The Spirocyclic Imine from a Marine Benthic Dinoflagellate, Portimine, Is a Potent Anti-Human Immunodeficiency Virus Type 1 Therapeutic Lead Compound Mar Drugs. 2019 Aug 24;17(9). PMID: 31450557

Useful Referece: Ref 1 describes infectious cDNA of HIV-1 which was used to construct expression system of this product.

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