HIV-1 Protease, functional

05-013 10 μg, 05-013-5 50 μg

Shipping and Storage: Shipped with dry-ice and stored at -80℃. When necessary, freezing (with liquid or ethanol in dry-ice) and thawing (in water at room temperature with shaking) should be done rapidly.

Product: Full-size functional recombinant HIV-1 protease purified from E. coli

Applications

1. Functional studies such as screening for inhibitors of HIV-1 protease for AIDS drug discovery
2. Antigen for anti-HIV 1 antibody for Western blotting and ELISA
3. Control as HIV 1 protease for SDS=PAGE

   Other applications have not been tested.

Stock solution: 0.2 mg /ml in 20 mM Tris, 20 mM MES, 0.2 M NaCl, 1 mMEDTA, 1 mM DTT, 10% Glycerol, pH 6.5

Reaction buffer: 20 mM Tris-HCl (pH 6.8), 1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 10% Glycerol

Background: HIV-1 protease is the aspartyl protease that mediates proteolytic cleavages of Gag and Gag-Pol polyproteins during or shortly after the release of the virion from the plasma membrane. Cleavages take place as an ordered, step-wise cascade to yield mature proteins. This process is called maturation. Displays maximal activity during the budding process just prior to particle release from the cell. Also cleaves Nef and Vif, probably concomitantly with viral structural proteins on maturation of virus particles. Hydrolyzes host EIF4GI and PABP1 in order to shut off the capped cellular mRNA translation. The resulting inhibition of cellular protein synthesis serves to ensure maximal viral gene expression and to evade host immune response.

Data Link UniProt P03367 (gag-pol), UniProt Q9YQ30 (HIV-1 Protease)

References : The HIV-1 strain and the recombinant protease has been described in the following 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 a*l "Overproduction of human immunodeficiency virus type I reverse transcriptase in Escherichia coli and purification of the enzyme" *Microbiol Immunol* **34**:509-521 (1990) PMID: 16991

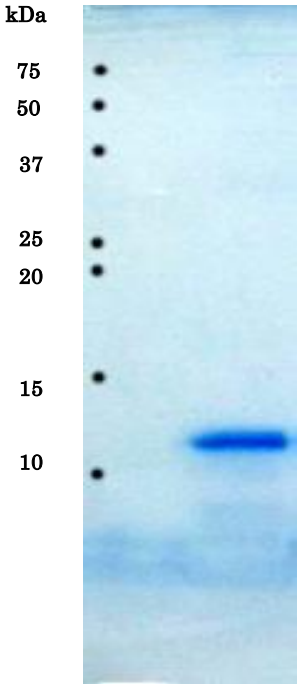

Fig.1 SDS-PAGE analysis of purified HIV-1 protease.

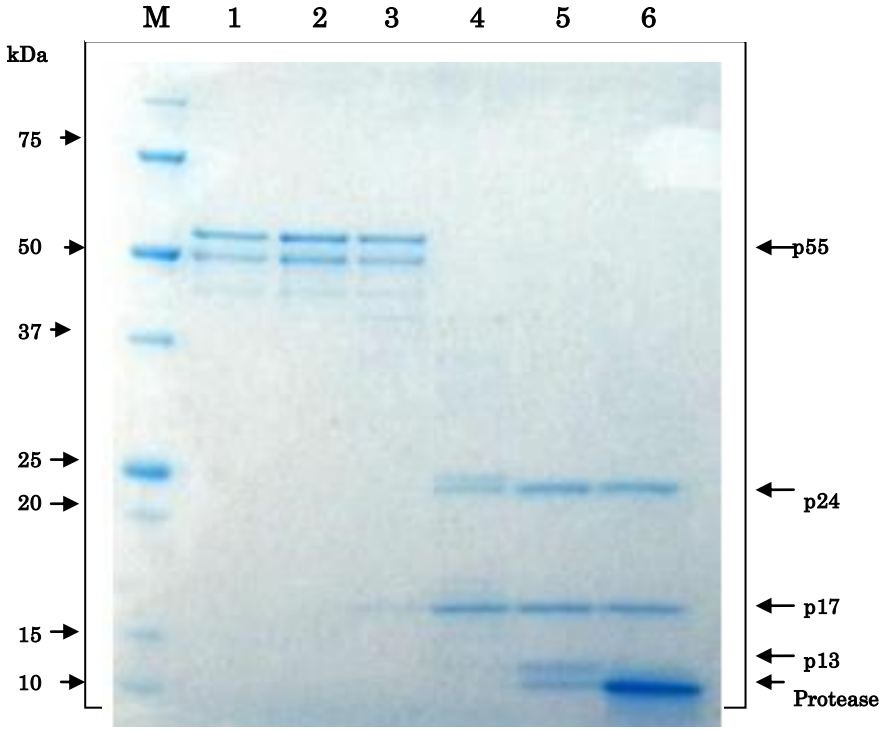

Fig.2 Proteolytic processing of HIV-1 Gag p55 proprotein by HIV-1 protease in vitro

As the substrate, recombinant Gag p55 (1 μg, BioAcademia 05-009) was used in 20 μl reaction volume. The reaction was carried by incubating at 37℃ for 3 h and stopped by adding SDS-PAGE sample buffer. 1; no protease, 2: 0.16 pg. 3; 1.6 pg. 4; 16 pg 5; 0.16 μg . 6; 1.6 μg protease. Note that two degradation bands are observed in the preparation of p55 substrate. In lane 4, p25 band is visible and in lane 5, p13 band is visible.

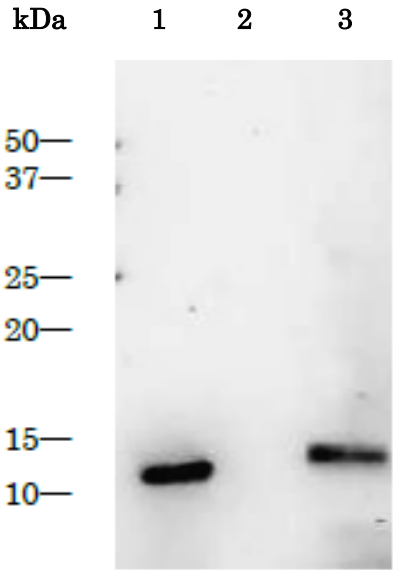

Fig.3 Purified HIV-1 protease as control antigen for Western blotting analysis with anti-HIV-1 protease antibody.

1: Purified HIV-1 protease (1 ng)

2: Extract of MT4 cells

3.: Extract of MT4 cells infected with HIV-1 (LAI strain)

The anti-HIV-1 protease antibody (BioAcademia 65-018) was used at 1/2,000 dilution.

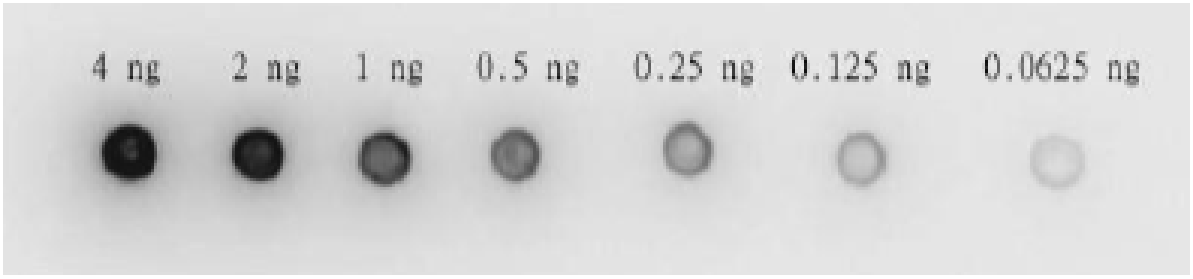

Fig.4. Dot blotting of HIV-1 protease by using anti-HIV-1 protease antibody.

Anti-HIV-1 protease antibody (BioAcademis 65-018) 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.

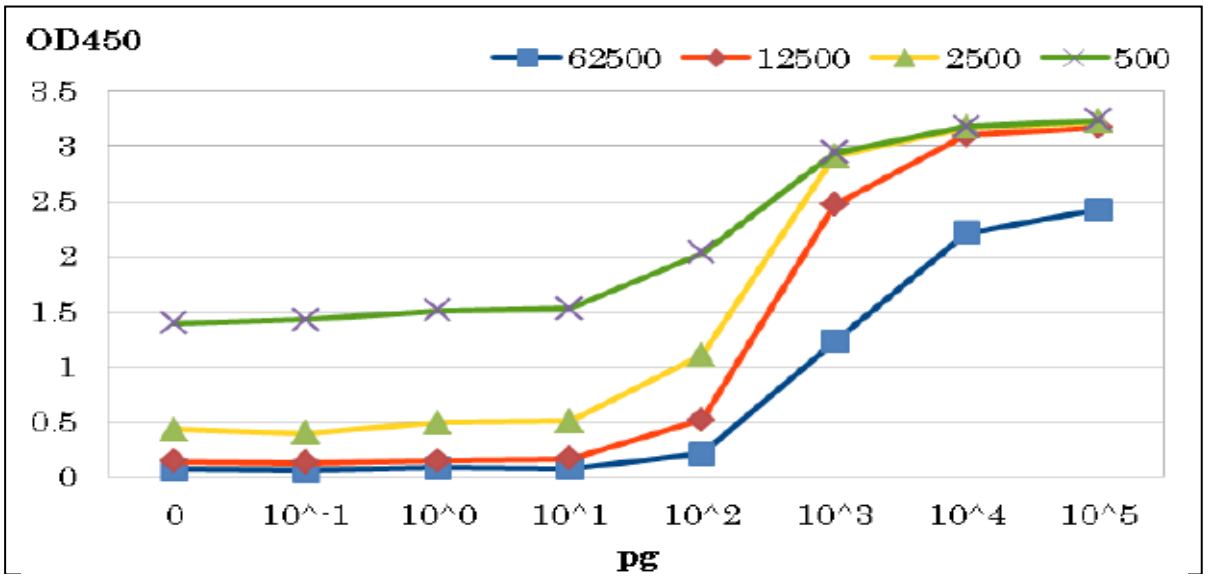

Fig.4 ELISA of HIV-1 protease with anti-HIV-1 protease antibody (BioAcademia 65-018).

The antibody was used at dilutions indicated above. Purified Protease was spotted on wells.