



NIH AIDS Reagent Program

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DATA SHEET

Reagent: pT328997_4

Catalog Number: 12465

Lot Number: 140261

Release Category: A

Provided: 5 µg of dried purified DNA stabilized in DNastable *PLUS*

Cloning Vector: See pNL4.3 GenBank accession AF324493.1 for the vector sequence. The vector contains an AmpR marker.

Cloning Site: Restriction maps match those of pNL4.3 with the exception of a novel Bal I site at nucleotide position 4552 and PflM 1 site at nucleotide position 5302 that were removed and a PflM 1 site added at nucleotide position 3491. The unique Apa I site upstream of protease at nucleotide position 2009 is used as a 5' restriction site; double-digestion with Apa I/Bal I generates a protease-deleted linearized vector. Inserts are amplified from clinically-derived viral cDNA using Pfu; Purified PCR products are digested and ligated into the vector using cycling T4 ligation, and the ligation product is grown in Stb13 E. coli in LB+carbenicillin, then plated and sequenced. The cloned region contains the entire protease open reading frame; the flanking regions cloned along with protease include 3' end of gag with the gag cleavage site, and the 5' end of RT.

GenBank: [KC109804](#)

Host Strain: Stb13 E coli for DNA and C8166/Sup T1 cell supernatant for Virus stock.

Description: This clone (clone T328997_4, PtID 3972) is part of a panel containing each of the canonical protease inhibitor-resistance pathways in clinically derived HIV-1 clones. In contrast to site-directed mutants, the mutations are present in their naturally occurring genetic contexts, which may include known accessory drug-resistance mutations, as well as changes at positions that are not currently known to be associated with drug resistance.

ALL RECIPIENTS OF THIS MATERIAL MUST COMPLY WITH ALL APPLICABLE BIOLOGICAL, CHEMICAL, AND/OR RADIOCHEMICAL SAFETY STANDARDS INCLUDING SPECIAL PRACTICES, EQUIPMENT, FACILITIES, AND REGULATIONS. NOT FOR USE IN HUMANS.

Special Characteristics: As the clones are also infectious and replication-competent, they can be used for in vitro susceptibility testing of new protease inhibitors (PIs). PIs that are active against these clones are likely to retain activity against the most clinically relevant, or possibly all, protease inhibitor-resistant variants. Researchers can also create their own recombinant viruses using the pNLPFB vector. A protocol is available upon request (please contact Shafer Lab)

[Plasmid map and sequence file lot 140261](#)

This clone is available individually, or as part of [Cat #12740 Panel of Multi-Protease Inhibitor Resistant Infectious Molecular Clones](#)

This reagent is currently being provided as dried purified DNA stabilized in DNastable *PLUS*. Please see the notice for additional information and the protocol for reconstitution of dried DNA reagents. [Dried DNA Notice](#)

Recommended Storage: Keep the reagent at room temperature in a dry storage cabinet or in a moisture barrier bag.

Contributor: Robert Shafer

References: Prototypical Recombinant Multi-Protease-Inhibitor-Resistant Infectious Molecular Clones of Human Immunodeficiency Virus Type 1 Vici Varghese, Yumi Mitsuya, W. Jeffrey Fessel, Tommy F. Liu, George L. Melikian, a David A. Katzenstein, Celia A. Schiffer, Susan P. Holmes, and Robert W. Shafer. *Antimicrob Agents Chemother.* 2013 September; 57(9): 4290–4299. doi: 10.1128/AAC.00614-13. [PMID: 23796938](#).

NOTE: Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH from Dr Robert Shafer: Cat# 12465, pT328997_4" Also include the reference cited above in any publications.

Last Updated: June 28, 2017

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