NIH AIDS Reagent Program
20301 Century Boulevard
Building 6, Suite 200
Germantown, MD 20874
USA

Phone: 240 686 4740
Fax: 301 515 4015
aidsreagent.org

DATA SHEET

Reagent:	p1556/W1235426-1
Catalog Number:	11849
Lot Number:	4 100207
Release Category:	A
Provided:	5 µg purified plasmid DNA, at 1 µg/µL in TE.
Cloning Vector:	pNL4.3ΔIN; 14.8kb.
Cloning Site:	HIV-1 Integrase, 5′ PacI site (4127) to 3′ EcoRI site (5743) in NL4-3; Insert is 1616bp.
GenBank:	See table.
Host Strain:	STBL3 E.coli (Grown in LB + 100µg/mL carbenicillin)
Description:	This clone is part of a panel containing each of the canonical raltegravir-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. As the clones are also infectious and replication-competent, they can be used for in vitro susceptibility testing of new integrase inhibitors (INIs). INIs that are active against these clones are likely to retain activity against the most clinically relevant, or possibly all, raltegravir-resistant variants. Researchers can also create their own recombinant viruses using the pNL4.3ΔIN vector. A protocol is available upon request (please contact Shafer Lab).
Special Characteristics:	The vector contains an AmpR marker; see pNL4.3 GenBank accession AF324493.1 for the vector sequence. Restriction maps match those of pNL4.3 with the exception of a novel PacI site upstream of integrase, generated by silent mutation at position 4127 (ATA->ATT). The unique EcoRI site downstream of integrase at position 5743 is used as a 3' restriction site; double-digestion with PacI/EcoRI generates an IN-deleted linearized vector. Inserts are amplified from clinically-derived viral cDNA using Pfu; second round PCR primers contain the PacI and EcoRI sites. Purified PCR products are digested and

ligated into the vector using cycling T4 ligation, and the ligation product is grown in Stbl3 E. coli in LB+carbenicillin, then plated and sequenced. The cloned region contains the entire integrase open reading frame; the flanking regions cloned along with integrase include vif, the 5' end of vpr, and the 3' end of RnaseH.

TABLE 1. Raltegravir-resistant HIV-1 Integrase Panel.
Sequences Info. Click here to view the sequences

Recommended Storage: Keep at -20°C or lower. Avoid freeze-thaw cycles as reagent degradation may result.

Contributor: Dr. Robert Shafer and Elizabeth Reuman, M.S.

References:

Panel of Prototypical Raltegravir-Resistant Infectious Molecular Clones in a Novel Integrase-Deleted Cloning Vector. Elizabeth C. Reuman, Michael H. Bachmann, Vici Varghese, W. Jeffrey Fessel, and Robert W. Shafer. *Antimicrob. Agents Chemother.* 2010;54 934-936.

NOTE: Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: (specify clone) from Dr. Robert Shafer and Elizabeth Reuman, M.S." Also include the references cited above in any publications.

Last Updated: February 13, 2015