

Product Information Sheet for HRP-12740

Panel of Multi-Protease Inhibitor Resistant Infectious Molecular Clones

Catalog No. HRP-12740

Lot No. 70055167

For research use only. Not for use in humans.

Contributor:

Dr. Robert Shafer, Professor, Division of Infectious Diseases, Department of Medicine, Stanford University, Stanford, California, USA

Manufacturer:

NIH HIV Reagent Program

Product Description:

HRP-12740 is a panel of 14 human immunodeficiency virus type 1 (HIV-1) infectious molecular clones. Clones in this panel contain each of the canonical protease inhibitor-resistance pathways in clinically derived HIV-1 in a pNL4.3 vector backbone (GenBank: AF324493).^{1,2}

Restriction maps of the clones in this panel 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 the protease at nucleotide position 2009 is used as a 5' restriction site; doubledigestion with Apa I/Bal I generates a protease-deleted linearized vector. Inserts were amplified from clinicallyderived viral cDNA using Pfu. Purified PCR products were digested and ligated into the vector using cycling T4 ligation. The cloned region contains the entire protease open reading frame; the flanking regions cloned along with protease include the 3' end of gag with the gag cleavage site, and the 5' end of RT. Plasmids can be propagated in STBL2 cells and grown at 37°C in the presence of ampicillin. Larger plasmids may benefit from growth at 30°C. These constructs may also be grown in other competent cells. Information on each molecular clone in this panel is shown in Table 1.1,2

In contrast to site-directed mutants, the mutations in each clone 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 these 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, PI-resistant variants. Researchers can also create their own recombinant viruses using the pNLPFB vector. A protocol is available upon request (please contact Shafer Lab).

Material Provided:

HRP-12740 is a set of 14 vials, each vial containing approximately 5 to 10 micrograms of dried, purified DNA stabilized in DNAstable®Plus. The patient identification number is also provided. Please see the protocol for reconstitution of dried DNA reagents on the NIH HIV Reagent Program webpage.

Packaging/Storage:

HRP-12740 was packaged aseptically in screw-capped plastic cryovials. The product should be stored at room temperature in a dry storage cabinet or in a moisture barrier bag.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH HIV Reagent Program, NIAID, NIH: Panel of Multi-Protease Inhibitor Resistant Infectious Molecular Clones, HRP-12740, contributed by Dr. Robert Shafer."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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NIH HIV Reagent Program

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References:

- Varghese, V., et al. "Prototypical Recombinant Multi-Protease-Inhibitor-Resistant Infectious Molecular Clones of Human Immunodeficiency Virus Type 1." <u>Antimicrob</u> <u>Agents Chemotherapy</u> 57 (2013): 4290-4299. PubMed: 23796938.
- 2. HIV Drug Resistance Database

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Table 1: Panel of Multi-Protease Inhibitor Resistant Infectious Molecular Clones

Catalog	Lot Number	Patient ID	Clone Number	GenBank
Number		Number		
ARP-11800	100161	38129	F719105_FY4	KC109813
ARP-11801	100164	794	H742358_H3	KC109799
ARP-11803	110241	634	F190755_3	GQ213968
ARP-11804	140257	4307	W37096_1	KC109805
ARP-11805	110243	1319	V38886_2	KC109800
ARP-11806	110244	18369	V48334_2	KC109811
ARP-11807	110245	27759	M135498_1	KC109812
ARP-11808	110246	1556	T450023_2	KC109803
ARP-11809	110247	1329	V16970_2	KC109801
ARP-12464	140260	2213	V20742-4	KC109814
ARP-12465	140261	3972	T328997_4	KC109804
ARP-12466	140262	6585	V42613_1	KC109808
ARP-12467	140263	7932	V57964-4	KP025970
ARP-12468	140264	14311	F160398_4	KC109810