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SUPPORTING INFECTIOUS DISEASE RESEARCH

Human Immunodeficiency Virus Type 1 (HIV-1) NL4-3 ΔEnv Vpr Luciferase Reporter Vector (pNL4-3.Luc.R-E-)

Catalog No. HRP-3418

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

HRP-3418 is a human immunodeficiency virus type 1 (HIV-1) NL4-3 luciferase reporter vector that contains defective Nef, Env and Vpr genes. To generate this reporter vector, a frameshift near the 5'-end of env was introduced by using T4 DNA polymerase to fill in the Ndel site (nucleotide 5950) of pNL4-3; this frameshift renders the clone Env deficient. A firefly luciferase gene was then inserted into the nef by removing the BamHI (nucleotide 8021) to XhoI (nucleotide 8443) fragment of pHXB-Luc and ligating it to the same sites in env-deficient pNL4-3. Another frameshift was introduced into vpr by filling in the Af/II site (nucleotide 5180) corresponding to amino acid 26.^{1,2,3} HRP-3418 is competent for a single round of replication and requires co-transfection with an Env expression vector to produce infectious virus. The beta-lactamase gene, bla, provides transformant selection through ampicillin resistance in Escherichia coli (E. coli). The plasmid size is 16,396 base pairs. The plasmid sequence and map are provided on the BEI Resources webpage.

<u>Note</u>: In this plasmid, Env was rendered deficient due to a small frameshift at the beginning of the *env*. As a result, there is a possibility of recombination with another *env* that could generate a viable virus. The sequences need to be tested prior to generating pseudoviruses of any kind with HRP-3418 and caution should be exercised.

Plasmids can be propagated in STBL2[™] cells and grown at 37°C. Larger plasmids may benefit from growth at 30°C. This construct may also be grown in other competent cells.

Material Provided:

Each vial contains plasmid DNA in TE buffer (10 mM Tris-HCI, 1 mM EDTA, pH 8). The DNA concentration and volume provided are shown on the Certificate of Analysis. The vial should be centrifuged prior to opening. <u>Note</u>: The contents of the vial should be used to replicate the plasmid in *E. coli* prior to expression studies.

Packaging/Storage:

HRP-3418 was packaged aseptically in cryovials. The product is provided frozen and should be stored at -20°C or colder immediately upon arrival. Freeze-thaw cycles should be avoided. Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Human Immunodeficiency Virus Type 1 (HIV-1) NL4-3 ΔEnv Vpr Luciferase Reporter Vector (pNL4-3.Luc.R-E-), HRP-3418."

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 (BMBL). Current Edition. Washington, DC: U.S. Government Printing Office.

Disclaimers:

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References:

 Connor, R. I., et al. "Vpr is Required for Efficient Replication of Human Immunodeficiency Virus Type-1 in Mononuclear Phagocytes." <u>Virology</u> 206 (1995): 935-944. PubMed: 7531918.

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- He, J., et al. "Human Immunodeficiency Virus Type 1 Viral Protein R (Vpr) Arrests Cells in the G2 Phase of the Cell Cycle by Inhibiting p34cdc2 Activity." <u>J. Virol.</u> 69 (1995): 6705-6711. PubMed: 7474080.
- Chen, B. K., et al. "Distinct Modes of Human Immunodeficiency Virus Type 1 Proviral Latency Revealed by Superinfection of Nonproductively Infected Cell Lines with Recombinant Luciferase-Encoding Viruses." J. Virol. 68 (1994): 654-660. PubMed: 7507183.

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