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DATA SHEET

Reagent: pRGH-ΔU3 (3')

Catalog Number: 12430

Lot Number: 130103

Release Category: E

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

Cloning Vector: pLAI

Cloning Site: The gag-enhanced green fluorescent protein (eGFP) fragment was inserted (ApaI-BssHII) into pLAI to create pLAI-Gag-iGFP. The cytomegalovirus-mCherry (CMV-mCherry) cassette was inserted into pLAI-Gag-iGFP using BpI-XhoI sites.

Host Strain: DH10B, or DH5α. Grow at 30°C.

Description: The construct, pRGH-ΔU3 (3') (pRGH(LAI-gagiGFP-CMVmCherry-ΔEnv)-ΔU3(3'LTR)), is a LAI-based, single cycle HIV-1 reporter containing an LTR-driven gag-iGFP marker and a CMV-driven mCherry marker. It contains a 126 bp KpnI/SacI linker from pTY-EFeGFP (cat# 4828) that deletes 557 bp of the U3 region of the 3' LTR.

Special Characteristics: This is one of a set of four reagents that were designed to study latency. The base construct is double-labeled vector, Red-Green-HIV-1 (pRGH-WT cat# 12427). The pRGH construct is a LAI-based HIV-1 reporter containing an LTR-driven gag-iGFP marker and a CMV-driven mCherry marker. The other constructs are: pRGH-Integrase D116A (cat# 12428) and pRGH-ΔCMV (cat# 12429).

This reagent was designed as a U3 (3' LTR) deletion control vector for pRGH-WT. Infectious pseudotyped virions can be produced by co-transfecting HEK293T cells with this vector as well as an appropriate envelope expression vector. Reporter gene expression in infected cells (LTR-eGFP, CMV-mCherry) can be detected by western blotting, fluorescent microscopy, or flow cytometry.

[Contributor provided sequence information](#)

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.

[Contributor provided plasmid map](#)

[Plasmid map and sequence file lot 130103](#)

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:

Ivan Sadowski and Viviana Simon

References:

Dahabieh, M.S., Ooms, M., Simon, V., and Sadowski, I. (2013) A doubly fluorescent HIV-1 reporter shows that the majority of integrated HIV-1 is latent shortly after infection. *Journal of Virology* 87 (8): 4716-4727.

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Shaner, N. C., Campbell, R. E., Steinbach, P. A., Giepmans, B. N. G., Palmer, A. E., and Tsien, R. Y. (2004). Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. *Nat Biotech* 22, 1567-1572.

Peden, K., Emerman, M., and Montagnier, L. (1991). Changes in growth properties on passage in tissue culture of viruses derived from infectious molecular clones of HIV-1LA1, HIV-1MAL, and HIV-1ELI. *Virology* 185, 661-672.

Chang L-J, Urlacher V, Iwakuma T, Cui Y, Zucali J. Efficacy and safety analyses of a recombinant human immunodeficiency virus type 1 derived vector system. *Gene Ther* 6:715-728, 1999.

Iwakuma T, Cui Y, Chang L-J. Self-inactivating lentiviral vectors with U3 and U5 mutations. *Virology* 261:120-132, 1999.

Cui Y, Iwakuma T, Chang L-J. Contributions of viral splice sites and cis-regulatory elements to lentivirus vector function. *J Virol* 73:6171-6176, 1999.

Zolotukhin S, Potter M, Hauswirth WW, Guy J, Muzyczka N. A "humanized" green fluorescent protein cDNA adapted for high-level expression in mammalian cells. *J Virol* 70:4646-4654, 1996.

NOTE:

Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: pRGH-ΔU3 (3') (#12430) from Ivan Sadowski and Viviana Simon." Also include the references cited above in any publications.

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