Simian-Human Immunodeficiency Virus Infectious Molecular Clone SHIV.BG505.332N.375Y.dCT

Catalog No. HRP-20057

For research use only. Not for use in humans.

Contributor and Manufacturer:

Hui Li, M.D., Research Associate Professor, and George M. Shaw, M.D., Ph.D., Professor of Medicine, Division of Hematology/Oncology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Product Description:

HRP-20057 is a full-length molecular clone of infectious and replication-competent simian-human immunodeficiency provirus. This clone contains an amino acid residue at Env position 375 that supports virus entry and replication in primary rhesus CD4 T cells.[1,2] BG505.332N.375S represents wildtype BG505 (GenBank: KU958484) env sequence with the 332 glycan knocking-in mutation T332N.[1,2] SHIV.BG505.332N.375Y.dCT is an isogenic mutant of SHIV.BG505.332N.375S.dCT generated by changing wildtype BG505 Env375 residue from Ser to Tyr.[1,2] SHIV.BG505.332N.375Y.dCT showed increased infectivity and replication kinetics *in vitro* in Indian rhesus macaque CD4$^+$ T cells and *in vivo* in Indian rhesus macaques.[1,2] The plasmid encodes full-length, replication-competent SHIV in a pCR-XL-TOPO backbone. The kanamycin resistance gene, *aph*, provides transformant selection through kanamycin resistance in *Escherichia coli (E. coli)*. The resulting size of the plasmid is approximately 13,910 base pairs. The plasmid sequence and map are provided on the NIH HIV Reagent Program webpage.

Note: Bacterial transformation and large-scale preparation of plasmid DNA are tricky due to plasmid instability. The contributors have developed a protocol to prevent this by growing bacteria for shorter time periods and at lower temperatures (see Appendix I).

Material Provided:

Each vial contains plasmid DNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA). The DNA concentration and volume provided are shown on the Certificate of Analysis. The vial should be centrifuged prior to opening. Note: The contents of the vial should be used to transform the plasmid in *E. coli* prior to mammalian expression.

Packaging/Storage:

HRP-20057 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen and should be stored at -80°C or colder immediately upon arrival. Freeze-thaw cycles should be avoided.

Citation:

Acknowledgment for publications should read “The following reagent was obtained through the NIH HIV Reagent Program, NIAID, NIH: Simian-Human Immunodeficiency Virus Infectious Molecular Clone SHIV.BG505.332N.375Y.dCT, HRP-20057, contributed by Dr. Hui Li and Dr. George M. Shaw.”

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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References:

1. Li, H. and G. M. Shaw, Personal Communication.
2. Li, H., et al. "New SHIVs and Improved Design Strategy for Modeling HIV-1 Transmission, Immunopathogenesis, Prevention and Cure." J. Virol. 95 (2021): e00071-21. doi: 10.1128/JVI.00071-21. PubMed: 33658341.

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APPENDIX I: PROTOCOL FOR MAXIPREP OF SHIV PLASMID DNA

Note: SHIV plasmids can be unstable during their expansion in *E. coli*, possibly due to the duplicated 5' and 3' LTR sequences or other factors. To reduce the likelihood of deletions or other rearrangements during plasmid propagation, follow the following protocol.[1]

Day 1:

1. Transform 10 to 50 ng of SHIV plasmid DNA using MAX Efficiency™ Stbl2™ Chemically Competent cells (Invitrogen, Catalog No. 10268-019). Incubate and shake the cells at 30°C.
2. Spread 100 µL of the transformation culture onto an LB agar plate containing 50 µg per mL kanamycin.
3. Incubate the plate at 30°C overnight. The incubation time should be less than 22 hours.

Day 2:

1. In the afternoon, inoculate six colonies individually into 3 mL of LB media with 50 µg per mL kanamycin.
2. Incubate the six mini-cultures overnight at 30°C while gently shaking at 200 rpm. The incubation time should be less than 22 hours.

Day 3:

1. For each mini-culture, take 2 mL for DNA miniprep and save the leftover culture at 4°C.
2. Run 5 µL of the miniprep DNA on an 0.8% agarose gel. The plasmid DNA should migrate around 10 kb (1 kb DNA ladder, New England Biolab, Catalog No. N3232S).
3. Do not proceed to the next step if there is a DNA band migrating around 3 to 4 kb, because this indicates that the bacteria culture already contains deleted plasmids (generally the empty vector without insert). Repeat steps above until a satisfactory mini culture is obtained that shows all DNA to be around 10 kb.
4. For the DNA maxiprep using any standard protocol and kit, select a mini culture without plasmid deletion and transfer 200 µL into 200 mL LB media with 50 µg per mL kanamycin.
5. Incubate the Maxi culture overnight at 30°C while gently shaking at 200 rpm. The incubation time should be less than 22 hours.

Day 4:

1. Important Note: Prior to performing the DNA maxiprep on the 200 mL maxi culture sample, take 2 mL from the maxi culture and repeat the DNA isolation and gel electrophoresis to confirm that the plasmid DNA is 10 kb in size. Then, proceed with the maxiprep isolation of plasmid DNA of the 200 mL sample. Confirm by gel electrophoresis that the final maxi-prep DNA is 10 kb in length and confirm its integrity by DNA sequence analysis.

This plasmid DNA is then used to transfect 293T cells to generate infectious SHIV virus stocks. Each stock must then be tested for p27Ag concentration (~ 1,000 ng per mL), vRNA concentration (~ 10^{10} vRNA per mL) and infectious units measured on TZM-bl cells (~ 10^5 to 10^6 IU per mL). The values shown are typical values obtained in the contributor's lab.

NIH HIV Reagent Program
www.hivreagentprogram.org
E-mail: contact@HIVReagentProgram.org
Tel: **888-487-0727** | Fax: **703-365-2898**

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