

Plasmid Containing Vaccinia Virus, Western Reserve Genome, VAC(WR)-LoxP-GFP-BAC

Catalog No. NR-17604

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

The entire vaccinia virus (VACV) Western Reserve (WR) genome (~195 Kb) (NCBI Accession AY243312) with a green fluorescent protein (GFP) sequence and two loxP sites was cloned into a plasmid vector and grown in *Escherichia coli* DH10β cells as a bacterial artificial chromosome (BAC). The loxP sites serve to achieve circularization of the virus genome in a Cre-loxP-mediated recombination system. Infectious vaccinia virus can be generated by transfecting the VACV-BAC plasmid into mammalian cells that have been infected with a nonreplicating fowlpox helper virus.^{1,2}

NR-17604 has been qualified for use in bacterial transformations.

Material Provided:

Each vial contains approximately 100 uL of plasmid DNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.0). The concentration, in µg per mL, is shown on the Certificate of Analysis for each lot.

Packaging/Storage:

NR-17604 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -60°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

Functional Activity:

The generation of infectious VACV after mammalian cell transfections with NR-17604 has not been confirmed at BEI Resources. The presence of authentic VACV WR and GFP sequences in NR-17604 has been confirmed by PCR amplification and partial nucleotide sequencing of the plasmid insert. The sequence of the entire VACV WR genome has not been confirmed due to the large size of the insert.

Citation:

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: Plasmid Containing Vaccinia Virus, Western Reserve Genome, VAC(WR)-LoxP-GFP-BAC, NR-17604.”

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. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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NR-17604 is claimed in U.S. Patent Number 7,494,813 and the continuations, continuations-in-part, re-issues and foreign counterparts thereof. Commercial use requires a license

from the U.S. Government. For further information contact the Office of Technology Transfer, National Institutes of Health, 6011 Executive Boulevard, Suite 325, Rockville, Maryland 20852-3804, (301) 496-7057.

References:

1. Domi, A. and B. Moss. "Cloning the Vaccinia Virus Genome as a Bacterial Artificial Chromosome in *Escherichia coli* and Recovery of Infectious Virus in Mammalian Cells." Proc. Natl. Acad. Sci. USA 99 (2002) 12415-12420. PubMed: 12196634.
2. Domi, A. and B. Moss. "Engineering of a Vaccinia Virus Bacterial Artificial Chromosome in *Escherichia coli* by Bacteriophage Lambda-Based Recombination." Nat. Methods 2 (2005): 95-97. PubMed: 15782205.

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