

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

Catalog No. NR-51176

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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}

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 is shown on the Certificate of Analysis for each lot. The vial should be centrifuged prior to opening.

Packaging/Storage:

NR-51176 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 presence of authentic VACV WR and GFP sequences in NR-51176 has been confirmed by PCR amplification, partial nucleotide sequencing of the plasmid insert, restriction enzyme digestion and generation of infectious VACV after mammalian cell transfection with NR-51176. 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-51176.”

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-51176 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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