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SUPPORTING INFECTIOUS DISEASE RESEARCH

Plasmid pBART1G, for Transfection in *Plasmodium berghei*

Catalog No. MRA-1265

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

MRA-1265 is a Plasmodium berghei (P. berghei) adaptable transfection vector (pBAT), containing recyclable drug-selectable and GFP-expression cassettes. The drugselectable cassette consists of a fusion of human dihydrofolate reductase (hDHFR) and yeast Fcu (cytosine deaminase-uracil phosphoribosyl transferase) under the control of *P. berghei* dihydrofolate reductase-thymidylate synthetase (PbDHFR-TS) 5' and 3' untranslated regions (UTRs).^{1,2} hDHFR confers resistance to pyrimethamine and WR99210, allowing positive selection of successfully transfected parasites. Yeast Fcu confers sensitivity to 5-fluorocytosine allowing recycling of the drug-selectable cassette. The tag sequence consists of a gene encoding the red fluorescent protein mCherry and a triple c-Myc epitope tag both preceded by a linker sequence and followed by the P. berghei dihydropterin pyrophosphokinase-dihydropteroate synthetase (PPPK-DHPS) 3'UTR. The high-expressing green fluorescent protein (GFP) cassette allows fluorescence microscopy analysis of life and fixed mutant parasites in all parasite life cycle stages. In pBART1G, also known as JX099569) the PbHSP70 5'UTR is pBART (GenBank: duplicated which, following recombination, results in the excision of the drug-selectable and GFP cassettes.^{1,2} The plasmid has 9834 base pairs and contains an ampicillin resistance marker. The complete plasmid sequence is provided on the BEI Resources webpage.

pBAT transfection vectors are useful in the genetic manipulation of *P. berghei,* such as gene deletion, disruption, tagging, and testing of promoter activity. MRA-1265 can also be used for cloning and shuttling of DNA fragments between the pBAT transfection vector set.¹

Material Provided:

Each vial of MRA-1265 contains approximately 500 ng of plasmid DNA in TE buffer (10 mM Tris-HCl and 1 mM EDTA). The concentration is shown on the Certificate of Analysis. The vial should be centrifuged prior to opening.

Packaging/Storage:

MRA-1265 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice 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: Plasmid pBART1G, for Transfection in *Plasmodium berghei*, MRA-1265, contributed by Taco W. A. Kooij."

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. <u>Biosafety in</u> <u>Microbiological and Biomedical Laboratories (BMBL)</u>. 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

Disclaimers:

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References:

- 1. Kooij, T. W. A., Personal Communication.
- Kooij, T. W. A., et al. "Expansion of Experimental Genetics Approaches for *Plasmodium berghei* with Versatile Transfection Vectors." <u>Mol. Biochem.</u> <u>Parasitol.</u> 185 (2012): 19-26. PubMed: 22705315.

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