

Toxoplasma gondii* Transformation Vector (pminCAT/HXGPRT-), Recombinant in *Escherichia coli

Catalog No. ARP-2851

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Lot No. 95031

Manufacturing Date: Unknown; before 1998

For research use only. Not for human use.

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Product Description:

ARP-2851 is an *Escherichia coli* (*E. coli*) DH5α stock expressing a transformation vector clone, pminCAT/HXGPRT-, containing a chloramphenicol acetyltransferase (CAT) coding sequence (GenBlock cassette; Pharmacia) flanked by 5' and 3' sequences derived from the *Toxoplasma gondii* dihydrofolate reductase-thymidylate synthase (*T. gondii dhfr-ts*) gene cloned into Bluescript pKS+ (Stratagene) polylinker *EcoRV-SmaI* sites. Both *EcoRV-SmaI* sites were destroyed during the cloning process. A unique *BglII* site is located upstream of CAT, and a unique *PstI* site is downstream. A *T. gondii* hypoxanthine-xanthine-guanine phosphoribosyl transferase (HXGPRT) minigene containing 5' genomic flanking sequences fused to cDNA encoding isoform I was cloned into the polylinker *XhoI* site upstream of CAT but in the opposite orientation. The *XhoI* fragment also contains some polylinker sequences from pGEM (Promega).

ARP-2851 is suitable for transient expression of CAT in transfected *T. gondii* parasites and/or stable transformation of HXGPRT-deficient *T. gondii* host strains (ARP-2857 and ARP-2860). The HXGPRT gene can be removed as a ~ 2000 base pair *XhoI* fragment for use as a selectable marker in other vectors; clone pminiHXGPRT (ARP-2855) works slightly better for this purpose. Clone pminCAT/HXGPRT- is identical to pminCAT/HXGPRT+ (ARP-2850), except for the orientation of the HXGPRT-containing *XhoI* fragment. Clone pminCAT/HXGPRT- exhibits poorer transformation frequencies than ARP-2850, however, possibly because of the strong hairpin produced by the polylinker sequences.

Material Provided:

Each vial contains approximately 500 μL of *E. coli* DH5α with pminCAT/HXGPRT- in Luria Bertani (LB) broth supplemented with 10% glycerol.

Packaging/Storage:

ARP-2851 was packaged aseptically in 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.

Growth Conditions:

Media:

LB broth or agar
pminCAT/HXGPRT- contains the gene required for chloramphenicol resistance. The standard concentration of chloramphenicol in culture is 20 μg per mL.

Incubation:

Temperature: 37°C
Atmosphere: Aerobic

Propagation:

Incubate the tube, slant and/or plate at 37°C for 1 day.

Citation:

Acknowledgment for publications should read "The following reagent was provided by the NIH AIDS Reagent Program for distribution by BEI Resources, NIAID, NIH: *Toxoplasma gondii* Transformation Vector (pminCAT/HXGPRT-), Recombinant in *Escherichia coli*, ARP-2851, contributed by Dr. David S. Roos."

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/bmb15/index.htm.

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References:

1. Roos, D. S., et al. "Molecular Tools for the Genetic Dissection of the Protozoan Parasite *Toxoplasma gondii*." Methods Cell Biol. 45 (1994): 27-63. PubMed: 7707991.
2. Pfefferkorn, E. R. and S. E. Borotz. "*Toxoplasma gondii*: Characterization of a Mutant Resistant to 6-Thioxanthine." Exp. Parasitol. 79 (1994): 374-382. PubMed: 7957757.

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