

***Toxoplasma gondii* Low-Level
Pyrimethamine-Resistance Plasmid
(pDHFR-TSc3/M3), Recombinant in
*Escherichia coli***

Catalog No. ARP-2853

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Lot No. 95033

Manufacturing Date: Unknown; before 1998

For research use only. Not for human use.

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Product Description:

ARP-2853 is an *Escherichia coli* (*E. coli*) DH5α stock containing a plasmid, pDHFR-RSc3/M3 (also referred to as pDHFR-RSc3/T83N¹), which confers low-level resistance to pyrimethamine.^{1,2} The *Toxoplasma gondii* dihydrofolate reductase-thymidylate synthase (*T. gondii dhfr-ts*) minigene, with the 5' genomic flanking sequence fused to cDNA, was cloned into the pKS- polylinker *HindIII-EcoRI* sites. An additional *EcoRI* site occurs near the *dhfr-ts* junction. The vector *BamHI* site has been filled. The wild-type *dhfr-ts* coding sequence contains a Thr83(ACC)-Asn(AAC) mutation that confers low-level pyrimethamine resistance to transfected parasites. This mutation is analogous to Ser108-Asn in fansidar-resistant *Plasmodium falciparum*.^{1,2}

ARP-2853 is suitable for stable transformation of *T. gondii*. Transformants should be isolated at low frequency (-4), and will typically contain multiple chromosomal or episomal transgenes, with a copy number roughly proportional to selection pressure. Linked genes may be co-amplified along with the selectable marker.²

Note: This plasmid confers resistance to pyrimethamine in *T. gondii*, rendering traditional pyrimethamine/sulfa therapy ineffective. In the event of accidental infection, alternative treatment (e.g., clindamycin and sulfa) should be administered immediately. Transgenic parasites harboring pyrimethamine resistance genes are not to be introduced into animals.

Material Provided:

Each vial contains approximately 500 μL of *E. coli* DH5α with pDHFR-TSc3/M3 plasmid in Luria Bertani (LB) broth supplemented with 10% glycerol.

Packaging/Storage:

ARP-2853 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

pDHFR-TSc3/M3 contains the gene required for pyrimethamine resistance. The standard concentration of pyrimethamine in culture is 150 ng 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* Low-Level Pyrimethamine-Resistance Plasmid (pDHFR-TSc3/M3), Recombinant in *Escherichia coli*, ARP-2853, 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/bmbl5/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. Donald, R. G. and D. S. Roos. "Stable Molecular Transformation of *Toxoplasma gondii*: A Selectable Dihydrofolate Reductase-Thymidylate Synthase Marker Based on Drug-Resistance Mutations in Malaria." Proc. Natl. Acad. Sci. USA 90 (1993): 11703-11707. PubMed: 8265612.

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