b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-50128

Genomic DNA from *Trypanosoma brucei* subsp. *rhodesiense*, Strain KETRI 2537 (*in vitro* procyclic form)

Catalog No. NR-50128

Product Description: Genomic DNA was isolated from *Trypanosoma brucei (T. brucei)* subsp. *rhodesiense*, strain KETRI 2537 (*in vitro* procyclic form; available as BEI Resources NR-50075). Strain KETRI 2537 (available as BEI Resources NR-46436, bloodstream form) was originally isolated in Busoga, Uganda, in 1972. The bloodstream form was harvested from the blood of infected BALB/c mice and adapted to cell culture by BEI Resources and extracted to produce NR-50128.

Lot[1]: 63904786 **Manufacturing Date: 24NOV2015**

TEST	SPECIFICATIONS	RESULTS
Agarose Gel Electrophoresis	High molecular weight chromosomal DNA	High molecular weight chromosomal DNA (Figure 1)
Content by PicoGreen® Measurement	≥ 3 µg in 25 to 100 µL per vial	2.9 µg in 47 µL per vial (61 µg/mL)[2]
Genotypic Analysis Sequencing of ITS 1, 5.8S ribosomal RNA gene, ITS 2 (~ 290 base pairs) Serum resistance-associated gene (SRA) (~ 590 base pairs)	 Consistent with *T. brucei* Consistent with *T. brucei* subsp. *rhodesiense*	 Consistent with *T. brucei*[3] Consistent with *T. brucei* subsp. *rhodesiense*
PCR Assay of Extracted DNA ITS 1, 5.8S ribosomal RNA gene, ITS 2[4] SRA[5,6]	 ~ 1300 base pair amplicon ~ 600 base pair amplicon	 ~ 1300 base pair amplicon ~ 600 base pair amplicon
OD_{260}/OD_{280} Ratio	1.6 to 2.1	2.0
Protozoan Inactivation 10% of total yield plated on SDM-79 medium[7]	 No viable organisms detected	 No viable organisms detected

[1]NR-50128 was produced from a culture of NR-50075 lot 63901335 from which procyclic forms of the organism were harvested. Genomic DNA was extracted using proprietary technology. NR-50128 lot 63904786 is provided in 10 mM Tris·Cl, 0.5 mM EDTA, pH 9.
[2]The post-vial calculation determined that there are less micrograms of material in the vial than was expected based on the pre-vialing calculations.
[3]Also consistent with *T. evansi* and/or *T. equiperdum*, which are putative subspecies of *T. brucei* [Lun, Z. R., et al. "*Trypanosoma brucei*: Two Steps to Spread Out from Africa." Trends Parasitol. 26 (2010): 424-427. PubMed: 20561822.].
[4]PCR was performed as described in Agbo, E. C., et al. "Measure of Molecular Diversity within the *Trypanosoma brucei* Subspecies *Trypanosoma brucei brucei* and *Trypanosoma brucei gambiense* as Revealed by Genotypic Characterization." Exp. Parasitol. 99 (2001): 123-131. PubMed: 11846522.
[5]Primer sequences and conditions for PCR are available upon request.
[6]Radwanska, M., et al. "The Serum Resistance-Associated Gene as a Diagnostic Tool for the Detection of *Trypanosoma brucei rhodesiense*." Am. J. Trop. Med. Hyg. 67 (2002): 684-690. PubMed: 12518862.
[7]Incubated in SDM-79 medium (Life Technologies, custom order part number ME090164 P1) adjusted to contain 10% (v/v) heat-inactivated fetal bovine serum (HIFBS) and 7.5 µg/mL hemin for 14 days at 27°C in an aerobic atmosphere.

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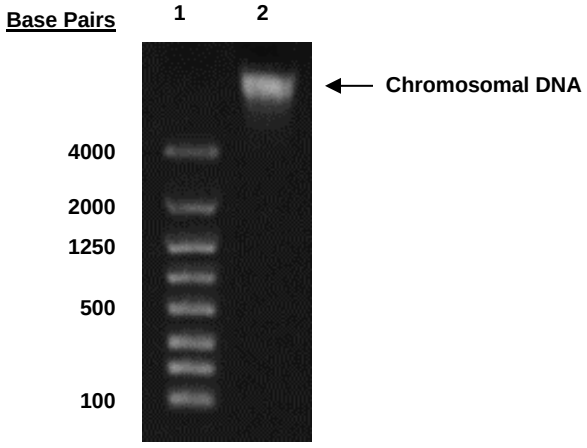
Figure 1: Agarose Gel Electrophoresis



Lane 1: Lonza FlashGel™ DNA Marker
Lane 2: ~ 184 ng of NR-50128

Date: 13 JAN 2017 **Signature:**

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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