Certificate of Analysis for NR-51498

Plasmid Containing 18S Ribosomal RNA Gene Fragment from *Cyclospora cayetanensis*

Catalog No. NR-51498

Product Description:

An approximately 1000 base pair fragment of the small subunit ribosomal RNA gene (18S rRNA gene) from *Cyclospora cayetanensis (C. cayetanensis)* was amplified by nested PCR and cloned into vector pCR™2.1-TOPO™ (Invitrogen™) and transformed into One Shot™ TOP10 *Escherichia coli (E. coli)* (Invitrogen™ C404010). The glycerol stock was grown in Luria-Bertani broth with 50 µg per mL ampicillin for 1 day at 37°C in an aerobic atmosphere, extracted using a EndoFree® Plasmid Maxi Kit (QIAGEN® 12362) and vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70021360 **Manufacturing Date: 05DEC2018**

TEST	SPECIFICATIONS	RESULTS
Confirmation of Insert by PCR[1]	~ 999 base pair amplicon	~ 1000 base pair amplicon
Genotypic Analysis Sequencing of insert (~ 1000 base pairs) 18S rRNA gene fragment (Figure 1)	≥ 99% sequence identity to *C. cayetanensis* 18S rRNA gene (GenBank: AF111183.1)	99.9% sequence identity to *C. cayetanensis* 18S rRNA gene (GenBank: AF111183.1)
Content by PicoGreen® Measurement	Report results	1.9 µg in 77 µL per vial (24.9 µg per mL)
Amount per Vial	0.2 to 3.5 µg	1.9 µg
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation (Post-Vial) Invitrogen™ One Shot™ TOP10 *E. coli*	≥ 100 colonies	≥ 100 colonies

[1]PCR amplification was performed as described in Murphy, H. R., S. Lee and A. J. da Silva. "Evaluation of an Improved U.S. Food and Drug Administration Method for the Detection of *Cyclospora cayetanensis* in Produce Using Real-Time PCR." J. Food. Prot. 80 (2017): 1133-1144. PubMed: 28590822. Primer sequences and conditions for PCR are available upon request.

Figure 1: Plasmid 18S rRNA Insert Sequence

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TGCTCGAGCG GCCGCCAGTG TGATGGATAT CTGCAGAATT CGCCCTTAAC CTGGTTGATC CTGCCAGTAG TCATATGCTT GTCTCAAAGA
TTAAGCCATG CATGTCTAAG TATAAGCTTT TATACGGTGA AACTGCGAAT GGCTCATTAA AACAGTTATA GTTTATTTGA TGGTCTCTTT
TACATGGATA ACCATGGTAA TTCTATGGCT AATACATGCG CACAGGCCTC CTTCTTTGGA GGGGCCGTGT TTATTAGATA CAAAACCAAC
CCACTTTGTG GAGCCTTGGT GATTCATAGT AACCGAACGG ATCGCATTTG GCTTTTGCCG GCGATAGATC ATTCAAGTTT CTGACCTATC
AGCTTTCGAC GGTAGGGTAT TGGCCTACCG TGGCATTGAC GGGTAACGGG GAATTAGGGT TCGATTCCGG AGAGGGAGCC TGAGAAACGG
CTACCACATC TAAGGAAGGC AGCAGGCGCG CAAATTACCC AATGAAAACA GTTTCGAGGT AGTGACGAGA AATAACAATA CAGGGCATTT
AATGCTTTGT AATTGGAATG ATAGGAATTT AAAATCCTTC CAGAGTAACA ATTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGGTAATTCC
AGCTCCAATA GTGTATATTA GAGTTGTTGC AGTTAAAAAG CTCGTAGTTG GATTTCTGTC GTGGTCATCC GGCCTTGCCC GTAGGGTGTG
CGCCTGGGTT GCCCGCGGCT TTCTTCCGGT AGCCTTCCGC GCTTCGCTGC GTGCGTTGGT GTTCCGGAAC TTTTACTTTG AGAAAAATAG
AGTGTTTCAA GCAGGCTTGT CGCCCTGAAT ACTGCAGCAT GGAATAATAA GATAGGACCT TGGTTCTATT TTGTTGGTTT CTAGGACCGA
GGTAATGATT AATAGGGACA GTTGGGGGCA TTCGTATTTA ACTGTCAGAG GTGAAATTCT TAGATTTGTT AAAGACGAAC TACTGCGAAA
GCATTTGCCA AGGATGTTTT CATTAATCAA GAACSMCART AGGGGGTTTG AAGACGAAGG GCGAATTCCA GCACAC
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/Sonia Bjorum Brower/
Sonia Bjorum Brower 13 SEP 2022
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