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Certificate of Analysis

Cilia-Associated Respiratory Bacillus

PCR Positive Control

Catalog Number: PCRPC118

Date: November 2016

Volume: 250 µl, 250 PCR Reactions

DNA Concentration: 150 ng/µl in TE buffer. Use 0.5 to 1.0 µl per PCR reaction.

Specific Activity: At least 500 copies per µl.

Storage conditions: -20° C to -70° C

Cilia-associated respiratory (CAR) bacillus: Is an unclassified, gram negative, filamentous ciliated bacterium that was tentatively designated in 1985 as one of the agents that cause chronic respiratory diseases in laboratory rodents.

Typical PCR reaction: Total volume 50 µl, 25 µl water, 5 µl 10x PCR Buffer, 2 µl 50

mM MgSO₄, 1 µl 10 mM dNTP mix, 1 µl 10 µM Forward Primer, 1 µl 10 µM Reverse Primer, DNA 0.5 to 1 µl, add water to 49.5 µl, and add last 0.5 µl *Taq* Polymerase. Mix, spin down gently, and put tubes in thermal cycler. Alternatively, a master mix including everything except some water and DNA may be used.

Typical Thermal Cycler Program:

- | | | |
|----|----------|----------|
| 1. | 94°C | 2 min |
| 2. | 94°C | 15 sec |
| 3. | 55°C | 30 sec |
| 4. | 0.5°/sec | to 68°C |
| 5. | 68°C | 2 min |
| 6. | Go to 2 | 35 times |
| 7. | 72°C | 5 min |
| 8. | 4°C | forever |
| 9. | End | |

PCR Primers: FOR 5'-GAG TTT GAT CCT GGC TCA G-3'

REV 5'-AGA AAG GAG GTG ATC CAG CC-3'

References: Kawano, A., *J. Vet. Med. Sci.* **62:7**, 797-800, 2000.

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