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Certificate of Analysis

Mycoplasma pulmonis

PCR Positive Control

Catalog Number: PCRPC110

Date: August 2012

Volume: 100 µl, 100 PCR Reactions

DNA Concentration: 200 ng/µl of DNA 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

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'-AGC GTT TGC TTC ACT TTG AA-3'

REV 5'-GGG CAT TTC CTC CCT AAG CT-3'

References: Kuppeveld, F., App. Environ. Microbiology **58:8**, August 1992, pages 2606-2615.

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