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Certificate of Analysis

Reovirus

PCR Positive Control

Catalog Number: PCRPC136

Date: August 2012

Volume: 250 μ l

RNA Concentration: 200 ng/ μ l in TE buffer. Use 0.5 to 1.0 μ l per PCR reaction.

Specific Activity: At least 500 copies per μ l.

Storage conditions: -20° C to -70° C.

First Strand Reaction: can be primed with anti-sense REV oligo at 1 μ l of a 1 μ M solution.

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

mM MgSO₄, 1 μ l 10 mM dNTP mix, 1 μ l 10 μ M Forward Primer, 1 μ l 10 μ M Reverse Primer, DNA 0.5 to 1 μ l, add water to 49.5 μ l, and add last 0.5 μ l *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'-GCT ATT TTT GCC TCT TCC C-3'

REV 5'-GAT GAA TGG AGC CTG TCC C-3'

FIRST STRAND 5'-CTA CTT ACC TCG GAC AGG G-3'

References: Leary, TP, J. Clinical Microbiology **40:4**, April 2002, pages 1368-1375.

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