**S1 Table.** **Reagents and thermocycling conditions used for two-step reverse-transcription polymerase chain reaction (RT-PCR) for amplification of influenza B PB1, PB2, PA, HA, NP, NA, MP and NS genes.**

1. Reagents and their volumes used for reverse transcription (RT) step.

|  |  |
| --- | --- |
| **Reagent** | **Vol. (1X), μl** |
| Universal Primer (Buni11W) | 4.5 |
| Template (RNA) | 10.0 |
| Water (Molecular Grade) | 12.6 |
| 5x First-Strand Buffer\* | 8.0 |
| 0.1M DTT\* | 2.0 |
| 100mM dNTP\* | 0.9 |
| SuperscriptTM III Reverse Transcriptase | 2.0 |
| **Total** | **40.0** |

\* InvitrogenTM, Life Technologies, USA

1. Thermocycling conditions for reverse transcription (RT) step.

|  |  |  |  |
| --- | --- | --- | --- |
| **Thermocycling steps** | **Temperature** | **Cycle** | **Time (Min)** |
| Denaturation and primer annealing | Mix primer and RNA. Incubate at 65°C. Then chilled on ice | 1 | 5 |
| Enzyme activation | 25°C | 1 | 5 |
| Extension | 50°C | 1 | 60 |
| Enzyme deactivation | 70°C | 1 | 15 |

1. Reagents and their volumes used for polymerase chain reaction (PCR) step.

|  |  |
| --- | --- |
| **Reagent** | **Vol. (1X), μl** |
| 10x PCR Buffer (15mM MgCl2)\* | 5.00 |
| dNTP mix (10mM of each) | 1.00 |
| Primer F | 2.00 |
| Primer R | 2.00 |
| HotStarTaq *Plus* DNA Polymerase (250 units) | 0.25 |
| RNase-free water | 35.75 |
| RT product | 4.00 |
| **Total** | **50.0** |

\* QIAGEN®, Germany

1. Thermocycling conditions for polymerase chain reaction (PCR) step.

|  |  |  |  |
| --- | --- | --- | --- |
| **Thermocycling steps** | **Temperature** | **Cycle** | **Time (Min)** |
| Initial activation | 95 | 1 | 5 |
| Denaturation | 94 | 40 | 0.5 |
| Annealing | 55 | 1 |
| Extension | 68 | 1.5 |
| Final Extension | 68 | 1 | 10 |