

**Genomic RNA from Usutu Virus, ENT MP 1626**

**Catalog No. NR-51220**

**Product Description:**

Genomic RNA was extracted from a preparation of cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells infected with Usutu virus (USUV), ENT MP 1626.<sup>1</sup>

**Lot: 70021063<sup>2</sup>**

**Manufacturing Date: 16APR2019**

TEST	SPECIFICATIONS	RESULTS
<b>Genotypic Analysis</b> Sequencing of species-specific region (~ 990 nucleotides)	≥ 98% identity with USUV	97.4% identity with USUV, ArD19848 (GenBank: KC754954.1) <sup>3</sup>
<b>Functional Activity by RT-PCR Amplification<sup>4</sup></b> Polyprotein gene	~ 1200 base pair amplicon	~ 1200 base pair amplicon (Figure 1)
<b>Pre-Vial Concentration by RiboGreen<sup>®</sup> Measurement (Viral, Cellular and Carrier)<sup>5</sup></b>	Report results	0.058 ng per 100 µL (0.0006 µg/mL)
<b>Estimated Amount per Vial<sup>5</sup></b>	Report Results	0.058 ng
<b>Virus Inactivation</b> 10% of total yield inoculated on Vero cells and evaluated for cytopathic effect <sup>1,6</sup>	No viable virus detected	No viable virus detected

<sup>1</sup>Vero cells: ATCC<sup>®</sup> CCL-81™

<sup>2</sup>Nucleic acid was extracted from a preparation of USUV, ENT MP 1626 (BEI Resources NR-51185 lot 70014222), using a QIAamp<sup>®</sup> Viral RNA Mini Kit (Qiagen 52906).

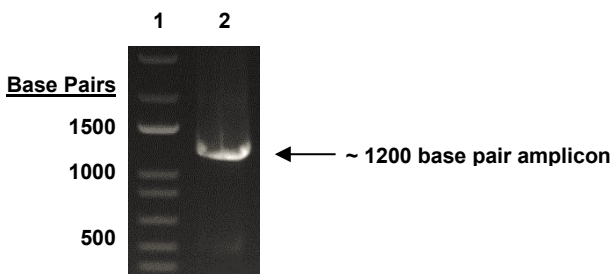
<sup>3</sup>Sequence information for USUV, ENT MP 1626 is not available in the NCBI database; nucleotide sequence obtained for NR-51220 lot 70021063 has 97.4% identity to USUV, ArD19848 (GenBank: KC754954.1) and 97.1% identity to USUV, SAAR 1776, the reference genome (GenBank: AY453412.1). Although the identity is less than 98%, the sequence obtained matched only Usutu viruses when BLAST was performed.

<sup>4</sup>Reverse transcription was performed using an iScript™ cDNA Synthesis Kit (Bio-Rad 170-8891) with 10 µL of NR-51220 in a 20 µL reaction; PCR was performed using iTaq™ DNA Polymerase (Bio-Rad 170-8870) with 5 µL of cDNA in a 50 µL reaction.

<sup>5</sup>Measurement is determined pre-vial prior to dilution due to the limit of detection of the quantification method

<sup>6</sup>Use of the QIAamp<sup>®</sup> Viral RNA Mini Kit has been demonstrated to consistently inactivate 100% of USUV as shown by the absence of cytopathic effect (CPE) after plating the entire extract on virus-susceptible cells.

**Figure 1: Functional Activity of NR-51220 by RT-PCR Amplification of Polyprotein Gene**



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder  
Lane 2: PCR product from 0.5 µL of NR-51220

/Heather Couch/

Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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