

**Usutu Virus, ENT MP 1626**

**Catalog No. NR-51185**

**Product Description:** Usutu virus (USUV), ENT MP 1626 was isolated from a mosquito (*Mansonia aurites*) pool in Zika Forest, Entebbe, Uganda in October 1962. Vial contains cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells<sup>1</sup> infected with USUV, ENT MP 1626.

**Passage History:** SM5V1/V2 (Prior to deposit at BEI Resources/BEI Resources); SM = Suckling mice; V = Vero E6 cells<sup>1</sup>

**Lot<sup>2</sup>: 70014222**

**Manufacturing Date: 23MAY2018**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 cells	Cell rounding and sloughing	Cell rounding and sloughing
Sequencing of Species-Specific Region (~ 940 nucleotides)	Consistent with USUV	Consistent with USUV <sup>3</sup>
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in Vero E6 cells <sup>1</sup> by Cytopathic Effect	Report results	1.6 × 10 <sup>7</sup> TCID <sub>50</sub> per mL
Amplification of USUV Sequence by RT-PCR	~ 1110 base pair amplicon	~ 1110 base pair amplicon
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>6</sup> , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
<b>Mycoplasma Contamination</b> Agar and broth culture (14-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

<sup>1</sup>Vero E6: ATCC® CRL-1586™

<sup>2</sup>Grown in Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g/L of sodium bicarbonate (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 8 days at 37°C with 5% CO<sub>2</sub>

<sup>3</sup>Sequence information for USUV, ENT MP 1626 is not available in the NCBI database; nucleotide sequence obtained for NR-51185 lot 70014222 is 98% identical to Usutu virus isolate ArD19848, complete genome (GenBank: KC754954.1).

<sup>4</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>5</sup>7 days at 37°C and 5% CO<sub>2</sub>

<sup>6</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/  
Heather Couch

27 SEP 2018

Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

