

**Usutu Virus, SAAR 1776**

**Catalog No. NR-51184**

**Product Description:** Usutu virus (USUV), SAAR 1776 was isolated from a mosquito (*Culex univittatus*) in Ndumo, Natal, South Africa in January 1959. Each vial contains cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells<sup>1</sup> infected with USUV, SAAR 1776.

**Passage History:** SM7V1/V2 (Prior to deposit at BEI Resources/BEI Resources); SM = Suckling mice; V = Vero cells<sup>1</sup>

**Lot<sup>2</sup>: 70014388**

**Manufacturing Date: 09MAY2018**

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in Vero cells</b>	Cell rounding and detachment	Cell rounding and detachment
<b>Sequencing of Species-Specific Region</b> (~ 990 nucleotides)	≥ 98% identity with USUV, SAAR 1776 (GenBank: MF374485.1)	100% identity with USUV, SAAR 1776 (GenBank: MF374485.1)
<b>Titer by TCID<sub>50</sub> Assay<sup>3,4</sup> in Vero cells<sup>1</sup> by Cytopathic Effect</b>	Report results	1.6 × 10 <sup>8</sup> TCID <sub>50</sub> per mL
<b>Amplification of USUV Sequence by RT-PCR</b>	~ 1100 base pair amplicon	~ 1100 base pair amplicon
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>5</sup> , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Blood agar, 37°C, aerobic 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: ATCC® CCL-81™

<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 5 days at 37°C with 5% CO<sub>2</sub>

<sup>3</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>4</sup>10 days at 37°C and 5% CO<sub>2</sub>

<sup>5</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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09 OCT 2018

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