

## Human Enterovirus 71 (HEV-71), Tainan/4643/1998

**Catalog No. NR-471**

**Product Description:** Cell lysate and supernatant from human rhabdomyosarcoma (RD) cells<sup>1</sup> infected with HEV-71, Tainan/4643/1998.

**Lot<sup>2</sup>: 7746372**

**Manufacturing Date: 25JAN2007**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in RD Cells <sup>1</sup>	Report results	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody Assay Monoclonal antibody to HEV-71 <sup>3</sup>	Fluorescence observed	Fluorescence observed
Sequencing of a Strain-Specific Region (~ 530 nucleotides)	Report results	Identical to GenBank AF304458
RT-PCR Amplification of an Enterovirus-Specific Region	~ 680 bp amplicon	~ 680 bp amplicon
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in RD Cells <sup>1</sup>	Report results	1.6 x 10 <sup>8</sup> TCID <sub>50</sub> /mL
<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 Test Article nucleic acid	None detected None detected	None detected None detected

<sup>1</sup>RD cells: ATCC<sup>®</sup> CCL-136™ (Lot: 4083062)

<sup>2</sup>HEV-71, Tainan/4643/1998 was deposited by National Cheng Kung University, Tainan, Taiwan. NR-471 was grown from the deposited virus seed in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370) supplemented with 2% irradiated fetal bovine serum (Cambrex<sup>®</sup> 14-471F), 2 mM L-glutamine (Invitrogen™ 25030), and 1 mM sodium pyruvate (Invitrogen™ 11360) for 3 days at 37°C and 5% CO<sub>2</sub>.

<sup>3</sup>Millipore MAB979.

<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>6 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.

**Date:** 12 SEP 2007

**Signature:** Signature on File

**Title:** Technical Manager, BEI Authentication or designee

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